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STUDIES ON THE INACTIVATION OF THE TUMOR-INDUCING PRINCIPLE IN CROWN GALL

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(Received for publication July 16, 1948)

Normal plant cells may be completely and permanently modified by the brief and localized presence of the crown-gall bacterium in susceptible plant tissues. Following the action of the bacteria, the proliferation of altered plant cells becomes an automatic process which is independent of the growth restraining influences of the host and the continued presence of the inciting bacteria. Aside from its biological activity, little is known concerning the nature of the principle responsible for the transformation of normal plant cells to tumor cells. Recently, however, a method has been described (2) for studying the process of tumor inception and development in those plant species that will not tolerate high temperatures for periods sufficiently long to kill the inciting bacteria of the crown-gall disease. This method is based on a finding which demonstrated that, although a temperature of 32° C. completely and abruptly halts the process of the transformation of normal cells to tumor cells, that temperature does not exert an inhibitory influence on the factors responsible for the multiplication of the tumor cells after the cellular transformation has been fully accomplished. The crown-gall bacteria were found to multiply as well in the host at 32° C. as at 25° C. By the use of this procedure it was possible to define precisely the period in which the cellular transformation is accomplished in *Vinca rosea* L., the Madagascar periwinkle. The results obtained in these experiments confirmed earlier work (1) in showing that there is a lag of somewhat more than one day between inoculation of plants with bacteria and the consummation of the cellular transformation. The results indicated further that the bacteria were capable for the most part of bringing about the alteration of normal cells to tumor cells only during the four-day period immediately following the wounding of the

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host. This was true despite the fact that many virulent bacteria were in intimate contact with the host cells after the fourth day.

By use of this technique it appeared possible to define more adequately the conditions which are critical to the neoplastic alteration and to characterize certain properties of the transforming principle. It is with these phases of the crown-gall problem that the present paper is concerned.

Methods and Materials

In the experiments reported here *Kalanchoe daigremontiana* Hamet and Perrier was used as the test object throughout. This host was selected because it characteristically produces small plantlets at the edges of its leaves. When mature these plantlets drop from the leaves and if environmental conditions are favorable they develop into plants typical of the species. The propagation of unlimited numbers of genetically uniform plants was therefore possible. The plants were grown in 4-inch pots and used when about 3 inches tall. Despite the fact that genetically similar plants were used in the experiments, some variation in the response of the plants to bacterial infection was noted. Different portions of the same stem sometimes responded differently to the inciting agent as measured by the size of the resulting tumors. Plants treated under similar conditions also showed at times some variation in their response to infection. As a result each experiment was performed on at least three different occasions during a period of more than a year and representative data from these are presented in the tables that follow.

The virulent B2 strain of *Agrobacterium tumefaciens* (Smith and Town.) Conn was used as the test organism. A moderately heavy tap water suspension of a 24-hour culture was inoculated into the plants by means of the needle puncture method.

Thermostatically controlled glass chambers in which the temperature was maintained at 32-33.5° C. were used throughout the work. The chambers were placed in a greenhouse and protected from the direct rays of the sun by shading. The relative humidity of the chambers was about 90 per cent. When not in the chambers, the plants were held on a greenhouse bench at 25°-26° C.

All results unless otherwise noted were recorded 14-16 days after the plants had been placed at 32° C. for the final incubation at that temperature. The criterion used here to determine whether normal

plant cells had been transformed to tumor cells by the bacteria was based on the fact that a perceptible mass of tumor tissue resulted from the growth of those cells after a suitable period.

Experimental Results

Exploratory experiments demonstrated that tumors were produced on inoculated *Kalanchoe* plants at a temperature of 25° C. but not at 32° C. In its response to heat, therefore, this host behaved as did the periwinkle (2). Since the period during which normal cells are transformed to tumor cells had not yet been defined for *Kalanchoe*, it was necessary at the outset to determine the minimum period necessary for the bacteria to accomplish the cellular alteration at 25° C. as well as the maximum time following wounding in which tumors could be initiated in this host.

As in the earlier work, *Kalanchoe* plants were inoculated at exactly 24-hour intervals for a period of 5 days. In certain experiments inoculations were made at intervals of 30, 32, 34, and 36 hours. Following inoculation the plants were permitted to remain on a greenhouse bench at a temperature of about 25° C. Six days after the first inoculations were made all plants were placed in a chamber at 32° C. for a period of 16 days. This was done to halt the process of cellular transformation and to permit development of tumors from cells that had been altered during the incubation period at 25° C. The results of one typical experiment which are shown in Table 1 and Figure 1 demonstrate that the cellular alteration was not accom-

TABLE 1

Representative experiment showing the effect of time on tumor inception and development. Inoculated plants were held at 25° C. for periods indicated below. The plants were then placed and held at 32° C. to stop the process of cellular transformation and permit the development of altered cells into tumors.

Incubation period at 25° C.	Relative size of resulting tumors
24 hours	—
30 hours	—, occ. ±
32 hours	—, ±
34 hours	—, ±
36 hours	±
48 hours	+, ++, occ. +++
72 hours	++, +++
96 hours	++, +++

occ. = occasionally.

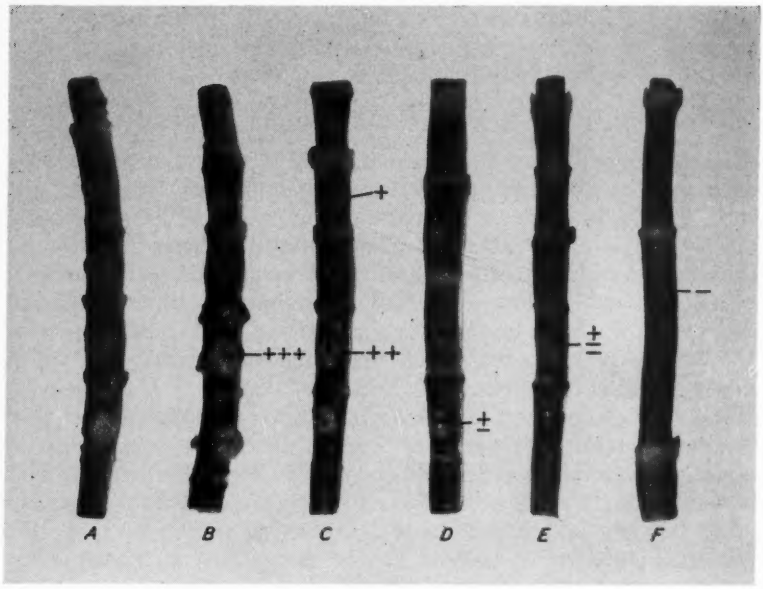

FIGURE 1

Kalanchoe stems inoculated with B2 strain of *Agrobacterium* tumefaciens. Following inoculation the bacteria were permitted to act on the host at 25° C. for (A) 96 hours, (B) 72 hours, (C) 48 hours, (D) 34 hours, (E) 30 hours, (F) 24 hours before the plants were placed at 32° C. to stop the process of cellular transformation and permit development of the tumors. Tumor size was graded as indicated in the Figure. (Photographs by J. A. Carlile.)

plished in 24 hours but was consummated during a 36-hour or longer incubation period at 25° C. Further experiments showed that the minimum period necessary for the bacteria to change normal cells to tumor cells was 32 ± 2 hours at 25° C.

In order to determine the maximum time in which tumors can be initiated in *Kalanchoe* after wounding, the host plants were inoculated with the crown-gall bacteria and placed immediately into a chamber at 32° C. At intervals of exactly 24 hours, plants were removed from the chamber and placed on a greenhouse bench for a period of 25 days. The results which are shown in Table 2 demonstrate that not only the percentage of tumors initiated but also

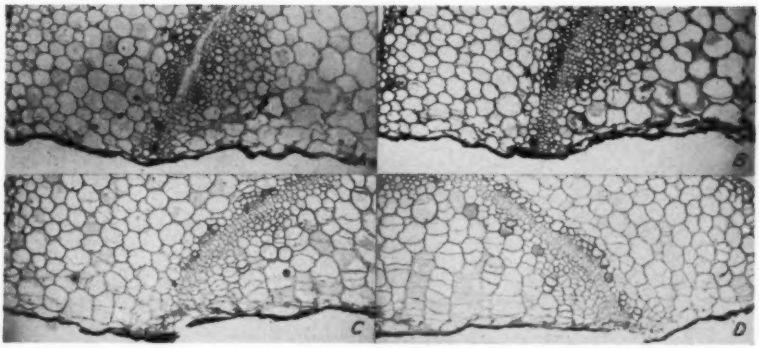

FIGURE 2

Four stages in the process of wound healing as it occurred in *Kalanchoe* at 25° C. Material was fixed (A) 24 hours, (B) 48 hours, (C) 72 hours, (D) 96 hours after wounding. Note that wound healing as determined by active cell division begins between the second and third day after wounding. (Photographs by J. A. Carlile.)

TABLE 2

One representative experiment showing the effect of incubation time at 32° C. on tumor inception and development. Plants were inoculated and placed immediately at 32° C. for periods indicated below. Following incubation at 32° C. the plants were placed and held at 25° C. The results were recorded 25 days after inoculation.

Incubation period at 32° C.	Relative size of resulting tumors	Percentage
24 hours	++, +++	100
48 hours	++, +++	100
72 hours	+, ++	67
	±	33
96 hours	±	82
	—	18
120 hours	±	15
	—	85

the size of the resulting tumors becomes progressively smaller between the second and fifth days after wounding. Plants held at 32° C. for 24 to 48 hours produced large tumors in all instances. On the other hand, about 85 per cent of the punctures were negative and the remainder gave rise to small tumors in those plants that had been held at 32° C. for a 5-day period. Thus we find an inception period which corresponds closely to that of the periwinkle (1, 2).

There is a lag of 32 ± 2 hours between inoculation and the beginning of transformation of normal cells to tumor cells. The host, for the most part, does not respond 5 days or more after wounding.

These results suggested that wound healing might serve as a limiting factor in the ability of the bacteria to convert normal cells to tumor cells. A histological study was therefore undertaken to determine the rate of wound healing in *Kalanchoe* at 25° C. and at 32° C. Needle punctures were made through internodes at 24-hour intervals for a period of 5 days. Six days after the first punctures were made the desired stem segments were placed in formalin-acetic acid-alcohol fixative, dehydrated and cleared, embedded in paraffin, sectioned and stained with dilute carbol fuchsin and orange G. Representative sections of the stages of wound healing as they occurred at 25° C. are shown in Figure 2. It is clear from these photographs that gross wound healing, as measured by the number of cell divisions following the puncture, becomes active between the second and third days after wounding. Thereafter the amount of healing becomes progressively greater until the process is complete. Wound healing in *Kalanchoe* progressed at about the same rate at 25° C. and at 32° C. The results reported in Table 1 demonstrate that normal cells are changed to tumor cells in 32 ± 2 hours when the plants are held at 25° C. following inoculation. Tumors of the size designated as ++ and +++ were initiated in 48 and 72 hours. This suggests that the process of cellular transformation is most active between the second and third days after inoculation. Thus in *Kalanchoe* it appears that it is just before or during the earliest stages of active wound healing that the cellular alteration is most readily accomplished.

Since the minimum period needed to accomplish the cellular transformation was found to be approximately 32 ± 2 hours at 25° C., an attempt was made to determine whether this period could be reduced appreciably by permitting the bacteria an initial incubation in the plants at 32° C., a temperature at which both the host and the organism develop well but at which tumors are not initiated. For these experiments an incubation period of 24 hours at 32° C. following inoculation was used. Preliminary studies had shown that this period approached the optimum for the experiments reported below. After the initial incubation at 32° C. the plants were placed at 25° C. for

TABLE 3

Plants were inoculated and placed immediately at 32° C. for 24 hours. Following incubation at 32° C. the plants were placed at 25° C. for the periods indicated below. After the desired incubation at 25° C. the plants were again placed and held at 32° C. to stop the cellular transformation process and permit the development of altered cells into tumors

Incubation period at 25° C.	Relative size of resulting tumors
6 hours	—
8 hours	—
10 hours	—, ±
12 hours	±
14 hours	+
16 hours	+
20 hours	++

the periods indicated in Table 3, after which time they were held at 32° C. to stop the process of cellular transformation and to permit development of the tumors. The results reported in Table 3 demonstrate that under the conditions of the experiment normal cells were not converted to tumor cells during a single exposure of 8 hours or less at 25° C. but were transformed in 10 hours or more. As the incubation period at 25° increased from 10 hours to 20 hours the relative size of the resulting tumors also increased. Thus, an exposure of 10 hours at 25° C. following an initial incubation of 24 hours at 32° C. yielded approximately the same results as did a total incubation time of 32 ± 2 hours at 25° C. These findings suggest, therefore, that the bacteria require an incubation period of roughly 20 to 24 hours for environmental adjustment plus an additional 10-hour period at 25° C. to produce the active principle in amounts necessary to transform normal to neoplastic cells. This does not mean, of course, that small amounts of the transforming principle may not be produced during the period of environmental adjustment.

It has been previously suggested (3) that the transforming principle either *1*) is very heat labile and is destroyed at 32° C. under the conditions that exist in the plants tested, or *2*) is rendered inactive by an inhibitor that is activated at or above 30° C., or possibly *3*) is not produced by the bacteria at the higher temperature. Experiments were next conducted, therefore, in an attempt to learn which, if any, of the three possibilities suggested above was the determining factor involved in the inability of the bacteria to accomplish the cellular transformation at 32° C. The problem was approached by

means of an experiment in which the plants were alternately subjected to temperatures of 25° C. and 32° C. following an initial incubation of inoculated plants for 24 hours at 32° C. A single 10-hour exposure at 25° C. was shown to be the minimum period in which the cellular transformation was accomplished under the conditions of these experiments. The alternate exposure times at 25° C. and 32° C. used here are listed in Table 4. In all instances a total of 30 hours at

TABLE 4

Plants were inoculated and placed immediately at 32° C. for 24 hours. Following incubation at 32° C. all plants were treated alternately at 25° C. and 32° C. for periods indicated below. Intermittent exposures totalling 30 hours at 25° C. were given in each instance. Following treatment all plants were held at 32° C.

Type of treatment given: Seven and one-half 4-hour periods at 25° C. alternated with *seven*	Relative size of resulting tumors	Calculated No. of units of transforming principle available
1-hour periods at 32° C.	++	23
2-hour periods at 32° C.	+	16
4-hour periods at 32° C.	—	2
Five 6-hour periods at 25° C. alternated with *four*		
1-hour periods at 32° C.	+++	26
2-hour periods at 32° C.	+, ++	22
4-hour periods at 32° C.	±, +	14
6-hour periods at 32° C.	—, ±	6

25° C. or three times the minimum 10-hour period necessary to convert normal to tumor cells at that temperature was given.

The results presented in Table 4 show that the transforming principle is cumulative. This is evidenced by the fact that, whereas a single exposure of 4 or 6 hours at 25° C. is inadequate in itself for the bacteria to bring about the cellular alteration, a series of exposures of these durations permits such an alteration. The data also demonstrate that a direct relationship exists between the size of the tumors produced and the total duration of exposure times at 32° C. alternated with exposures at 25° C. for an accumulated period of 30 hours at the lower temperature. For example, large tumors were produced when the bacteria were permitted to act for periods of 4 or 6 hours, totalling in each instance 30 hours at 25° C. when these periods were alternated with one-hour exposures at 32° C. As the intervals that the plants were held at 32° C. were increased in rela-

tion to the total time that they were held at 25° C., the size of the resulting tumors decreased. When the total exposure at 32° C. approached the exposure at 25° C. small tumors or no tumors at all were produced. The results suggest, therefore, that the activity of the transforming principle was destroyed at a temperature of 32° C. at roughly the same rate that this principle was produced by the bacteria during a comparable period at 25° C.

This finding suggests that the residual activity of the transforming principle can be computed on a semi-quantitative basis. For convenience in these calculations, 10 units of transforming principle are arbitrarily considered necessary to alter irreversibly normal *Kalanchoe* cells to tumor cells. A single 10-hour exposure period has been established as being the minimum time necessary to accomplish the cellular alteration under the conditions of the experiment. If it is assumed that the transforming principle is being produced at a constant rate by the bacteria after they have established themselves in the host, then it follows that one unit of transforming principle is produced every hour during the incubation at 25° C. The inactivation of this principle at 32° C. was found to be roughly equal to its production at 25° C. Thus for every unit produced at 25° C. in one hour, approximately one unit was destroyed at 32° C. in the same period. In the series of experiments shown in Table 4 in which 4-hour intervals at 25° C. were alternated with 1-hour intervals at 32° C., a total of 30 units was produced and 7 were destroyed leaving 23 units available for the cellular transformation. These 23 units are equal to somewhat more than twice the minimum of 10 units necessary to produce the irreversible cell change. The size of the tumors produced experimentally corresponded to a ++ tumor. Other results expressed in units are shown in Table 4. These data show that roughly 25 units or more of the transforming principle appear to be necessary to initiate tumors of the size designated as +++. The availability of 20 to 24 units resulted in ++ tumors, while 14 to 19 units gave a + reading. About 10 units are the minimum necessary to accomplish the irreversible cell change under the conditions of these experiments. These figures, of course, represent merely a rough approximation and are not to be taken as absolute values. It should be pointed out here that once the concentration of transforming principle necessary for the conversion of normal cells to tumor cells

is reached or passed, the reaction becomes irreversible and the resulting tumor cells develop into a neoplasm independently of the bacteria. In these experiments only the amount of cellular alteration that had been accomplished during a 30-hour period at 25° C. was considered.

If the results shown in Table 4 are compared with those in Table 3, it is found that a single exposure of 10 to 12 hours at 25° C. is sufficient to give a ± reading, while 14-hour and 20-hour periods permit the bacteria to initiate tumors of the size designated as + and ++, respectively. Thus, in this single exposure experiment, in which the number of hours of incubation at 25° C. is expressed directly in units, the results obtained correspond very well with the results found in the experiments in which the transforming principle was intermittently produced at 25° C. and either partially or wholly inactivated at 32° C.

The unit hypothesis was checked experimentally in another way. Instead of permitting the bacteria to act on the host for alternate periods at 25° C. and 32° C. for a total of 30 hours at the lower temperature, a series of shorter total exposures at 25° C. were used. In these experiments 4-hour periods at 25° C. were alternated with 1 and 2-hour periods at 32° C. The total exposures at 25° C. in each experiment are indicated in Table 5. The results of these experiments demonstrated again that the relative size of the tumors initiated corresponds within the limits of biological experimentation with the calculated number of units of transforming principle produced.

Discussion

An observation reported earlier demonstrated (2) that normal plant cells may be converted into tumor cells by the crown-gall bacteria at a temperature of 25° C. but not at 32° C. Once the cellular transformation has been fully consummated at 25° C., however, the tumor cells develop into a neoplastic growth at both temperatures. The bacteria and the host develop well at 32° C. By utilizing this finding, it was possible to define certain conditions which are critical to the neoplastic alteration.

The experiments reported here demonstrated that normal cells of the host plant *Kalanchoe daigremontiana* were converted into tumor cells in 32±2 hours at 25° C. The plants were for the most part no

TABLE 5

Plants were inoculated and placed immediately at 32° C. for 24 hours. Following incubation at 32° C. the plants were held alternately at 25° C. and 32° C. for periods indicated below. Following treatment all plants were held at 32° C.

No. of 4-hour periods at 25° C.	No. of alternate 1-hour periods at 32° C.	Total time at 25° C. in hours	Total time at 32° C. in hours	No. of available units	Results	No. of 4-hour periods at 25° C.	No. of 2-hour periods at at 32° C.	Total time at 26° C. in hours	Total time at 32° C. in hours	No. of available units	Results
2	1	8	1	7	—, occ. ±	2	1	8	2	6	—
3	2	12	2	10	—, ±	3	2	12	4	8	—, occ. ±
4	3	16	3	13	±, +	4	3	16	6	10	—, ±
5	4	20	4	16	±, +	5	4	20	8	12	±
6	5	24	5	19	+, ++	6	5	24	10	14	±, +
7	6	28	6	22	+, ++	7	6	28	12	16	±, ++
7.5	7	30	7	23	++	7.5	7	30	14	16	±, +

occ. = occasionally.

longer susceptible 5 days after wounding. When, however, the plants were inoculated with bacteria and held at 32° C. for 24 hours and then placed for as short a period as 10 hours at 25° C., the cellular transformation was accomplished. Periods of 8 hours or less at 25° C. following a 24-hour incubation period at 32° C. were inadequate to bring about the cellular alteration. In each instance described above a total of 30 to 34 hours is necessary to accomplish the alteration. These results suggest, therefore, that the bacteria require an incubation period of roughly 20 to 24 hours either at 25° C. or at 32° C. for environmental adjustment plus an additional 10-hour period at 25° C. to produce the transforming principle in the amounts necessary to bring about the cellular alteration. It has also been established from these results that a definite threshold concentration of the transforming principle is necessary to convert normal plant cells to tumor cells. Once this threshold is reached or passed the reaction becomes irreversible and the tumor cells develop into a neoplastic growth.

Three possibilities have previously been presented in an attempt to account for the inability of the crown-gall bacteria to alter normal plant cells to tumor cells at a temperature of 32° C. It was suggested (3) that either the transforming principle (*1*) is not produced by the bacteria at 32° C., or (*2*) is rendered inactive by an inhibitor that is activated at that temperature, or (*3*) is very heat labile and is destroyed at 32° C. under the conditions that exist in the plants tested.

It has not been possible to determine from the data presented here whether the transforming principle is produced by the bacteria at 32° C. Whatever the effect of this temperature on the ability of the organisms to produce this principle, the results nevertheless suggest that the transforming principle itself is inactivated at 32° C. This is shown by the fact that large tumors were produced when the bacteria were permitted to act for periods of 4 or 6 hours totalling in each instance 30 hours at 25° C. when these periods were alternated with one-hour exposures at 32° C. As the intervals that the plants were held at 32° C. were increased in relation to the time that they were held at 25° C. the size of the resulting tumors decreased. When the interval at 25° C. approached or equaled the interval at 32° C. small tumors or no tumors at all resulted. This suggests that the transforming principle was completely or almost completely inactivated under the

conditions of this experiment. If no inactivation of this principle had occurred at 32° C. the resulting tumors would all have been equal in size because a total of 30 hours at 25° C. was given in each instance. Other data reported here confirm this conclusion.

The question, however, as to whether the inactivation of the transforming principle at 32° C. was the result of the activation of an inhibitor at that temperature or whether the transforming principle itself is heat labile is impossible to answer from the data presented.

In postulating an inhibitor mechanism several assumptions have to be made to account for the observed results. It would have to be assumed, for example, that the inhibitor itself is free to act at 32° C. but that it is bound at 25° C. and as a result is rendered ineffective against the transforming principle at that temperature. This reaction would either have to be reversible or, if the substrate to which the inhibitor is bound is heat labile and is destroyed at 32° C., that material would have to be promptly replaced at 25° C. That this postulated inhibitor is not cumulative to any appreciable extent at 32° C. is evidenced from the fact that when plants were placed at 32° C. for 24 hours immediately after inoculation and then held at 25° C. the cellular alteration was accomplished in as short a period as 10 hours at the lower temperature. Thus, the incubation period at 32° C. for 24 hours plus 10 hours at 25° C. yielded the same result as did total incubations of 32 to 34 hours at 25° C. This demonstrates, therefore, that there was no appreciable accumulation of an inhibitor following a 24-hour incubation period at 32° C. While such an inhibitor system cannot at present be ruled out it appears that all the results obtained here can be explained more directly on the basis that inactivation at 32° C. is due to a thermal destruction of the transforming principle itself. Further work on this phase of the problem is now being carried out.

It has been indicated that in these experiments the tumor-inducing principle was inactivated at 32° C. at roughly the same rate that it was produced by the bacteria at 25° C. By shifting the balance in favor of production, on one hand, or inactivation on the other, it was possible to predict within the limits of biological experimentation the eventual outcome by merely adding the number of hours that the plants were held at 32° C. and subtracting this figure from the number of hours that the plants were alternately kept at 25° C. It could

be determined from the value received not only whether tumors would be initiated, but, if they were produced, the relative size of the resulting tumors could be predicted with some accuracy. The results suggest, furthermore, that the relative size of the resulting tumors, in the early stages of their development, appears to be dependent upon the amount of tumor-inducing principle available at the cellular alteration.

Summary

This investigation is based on the finding that normal *Kalanchoe* cells may be transformed to tumor cells by the crown-gall bacteria at a temperature of 25° C. but not at 32° C. Once the cellular transformation has been accomplished the tumor cells develop into a neoplastic growth at both temperatures.

The results obtained demonstrate that the minimum period necessary for the bacteria to alter normal cells to neoplastic cells is 32 ± 2 hours at 25° C. The plants were for the most part no longer susceptible 5 days after wounding. The period necessary for the bacteria to accomplish the cellular alteration could be reduced from 32 ± 2 hours to 10 hours at 25° C. when the plants were permitted an incubation period of 24 hours at 32° C. following inoculation.

A comparison of histological findings and plant inoculation studies indicated that it is before or during the early stages of active wound healing that normal cells are most readily converted into tumor cells.

The studies reveal, furthermore, that inactivation of the tumor-inducing principle itself occurs at 32° C. The possible nature of this inactivation is discussed.

The principle responsible for the alteration of normal cells to tumor cells was inactivated at 32° C. in these experiments at about the same rate that it was produced by the bacteria during a comparable period of time at 25° C. As a result of this finding a semi-quantitative method was developed which made it possible to predict within the limits of biological experimentation not only whether tumors would be initiated under a given set of experimental conditions, but, if they were produced, the relative size of the resulting tumors could be estimated in advance with some accuracy.

The relative size of the tumors appears to be dependent upon the amount of transforming principle available at the time of the cellular alteration.

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THE DEVELOPMENT OF THE SECRETORY CELLS OF RICINUS AND THE PROBLEM OF CELLULAR DIFFERENTIATION

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The origin of cellular diversity or differentiation in the tissues of multicellular plants is one of the fundamental problems of morphogenesis and is also, like the related processes in animals, of very great theoretical and practical significance. Even a casual study of the ontogenetic process conveys the fact that diverse types of cells, typical for each species, are formed; these cell types can be distinguished by their shape, walls, contents and physiological behavior and specificity. These differences among the originally similar elements of the ground tissue may appear early or late and do not occur at random, but in definite locations and patterns, such as the appearance of typical epidermal cells in the superficial layer or the endodermal cells at the boundary of cortex and stele. These two facts, first, that cells become unlike one another, second, that the differences do not occur at random, but in orderly patterns, are probably somehow closely related aspects or expressions of the still enigmatic mechanism of differentiation.

The fundamental nature of the factors controlling such orderly differentiation is generally recognized; their knowledge might indeed give us the clue to what is called the organization of living things. It is the more astonishing that relatively very little effort has been diverted toward the study of the mechanism of histological differentiation in plants. Even the grosser aspects and phases in the formation of many characteristic and frequently occurring plant cell types are only superficially known. The physiological character of a variety of specialized cells has not been adequately investigated and described. Only in a few cases have the external conditions been studied under which cells tend to differentiate in a specific manner. It would appear, therefore, essential to undertake anatomical, developmental

and biophysico-chemical studies of many forms of cellular differentiation and to study the conditions under which they occur, before any general theory of histological differentiation can be successfully developed.

The theories which have been put forward to explain the origin of cellular differentiation usually approach the problem from one of two angles. They either associate the orderly unfolding of cellular differences with genetically determined internal factors, residing and manifesting themselves in the nucleus or cytoplasm; or, they take into account known differential effects of environmental factors on potentially identical cytoplasmic units in different positions in the plant body. Only the surface of this problem has been touched, but there seems to be evidence that both these aspects are involved.

Let us consider briefly the first group of suggestions. Its most outstanding proponent was Weismann whose theory of a progressive sorting out at division of nuclear determinants put the case clearly, but this theory had to be rejected for various reasons. It would appear more helpful to assume that in some cases and at certain divisions by a yet unexplained mechanism specific *cytoplasmic* determinants become segregated, sorted out or quantitatively distributed in a differential manner. The frequent occurrence of differential cell divisions of polar character (cf. Sinnott and Bloch, 11, 12; Bloch, 3) as well as cytoplasmic stabilized changes in certain cells (cf. Bloch, 4) seem to support this idea.

On the other hand, the fact should not be overlooked that differential external conditions have a marked influence on the differentiation of many cells. This may be particularly well demonstrated in cells located at the surface of an organ. The cell wall differentiates here in a characteristic manner, and if the epidermal and subepidermal layers of cells are removed experimentally, including the layers of specialized cells, non-specialized cells of the ground tissue which come to be located in a corresponding position often develop, even without previous division, characteristic wall thickenings originally destined for the superficial cells now removed (Bloch, 1, 2). That both external and internal factors may combine in the determination of the type of cell to be formed, has been demonstrated in the case of polar, differential divisions in *Monstera deliciosa*. Unequal divisions occur in both the inner cortical and hypodermal cells

of the air root which result in formation of small cells. In the inner cortex, the small cells become trichosclereids; in the hypodermal layer, however, they develop into the physiologically specific "short" cells (Sinnott and Bloch, 12).

These brief remarks indicate that the diverse phases and aspects of cellular differentiation cannot be studied in all cells with the same advantage; it is evident that some cells are more suitable than others for the study of a specific aspect or phase of differentiation.

In all studies of histological differentiation the occurrence of *"differential"* cell divisions is of extreme interest. Many cell types make their appearance rather abruptly as a result of such differential divisions. It would seem that the greatly neglected study of these divisions is one of the most hopeful approaches to the problem of differentiation. Among the great variety of differential cell divisions are such different types as indefinitely dividing cells (e.g. apical cells and cambial initials), and cells dividing once only, giving rise to two cells, one of which generally remains unspecialized, while the other differentiates in a quite specific fashion (e.g. root hair cell division). Other cell types do not seem to differentiate predominantly as the result of such conspicuous differential division, but appear to develop specific properties in reaction to local external conditions operative in certain positions in the plant.

The present report deals with cells which belong to the category of so-called "idioblasts." These are cells of solitary nature since they are, as a rule, not associated with any particular tissue system, but occur more or less irregularly scattered within the ground tissue. Examples are: crystal cells, tannin cells, and various other kinds of secretory cells and sclereids. Although in the anatomical texts many types have been described, relatively little is known about their origin and mode of development. They have recently attracted the interest of investigators on account of their individualistic behavior. It has been shown, for example, that idioblastic sclereids may arise in a number of different ways; some arise as a result of a typical differential division, in the manner of root hairs (e.g. the above-mentioned trichosclereids of *Monstera*); others make their appearance in specific positions, for instance at the ends of foliar veins (e.g. in *Mouriria;* Foster, 7); still others seem to have no recognizable relation to adjacent cells (e.g. *Camellia;* Foster, 6).

The writer has investigated the secretory cells which occur in the seedling and mature plant of the castor bean, *Ricinus communis*. These cells, like crystal cells, tannin cells and oil cells, are cells of physiologically distinct character about which very little is known. During the course of the investigation it became evident that cells of this kind, if more fully understood, might give valuable information as to the factors controlling differentiation.

Ricinus contains at least three kinds of idioblasts, those containing crystals of calcium oxalate, tannin sacs and the secretory cells. The last-named have been described sometimes as tannin cells, sometimes as fat cells (cf. Solereder, 13; Scott, 10). Although they are clearly distinguishable from other cells, since they give the reactions of tannin and unsaturated fatty acids, their chemical nature has not yet been properly defined. Scott has described the general distribution of these cells in the various tissues of the plant; they occur in typical locations, in the epidermis of the leaf, along the veins of the leaves, at the leaf bases, in the stem cortex and pith, along vascular strands, and in the secondary phloem.

The present writer has briefly called attention to the peculiar developmental and morphogenetic properties of these cells (4). Contrary to the origin of most known specifically differentiated cells, they arise both very early (apical meristem) and very late (older internodes, secondary tissues, wound tissues). Furthermore, once differentiated, they show remarkable tenacity in retaining their specialized character along with the power of multiplication, under conditions where ordinarily plant cells dedifferentiate and become more or less non-specific. Secretory cells which divide thus produce only secretory cells, even under conditions of regenerative activity in old internodes. A more detailed study of their development and of the problems they pose has since been made and is here briefly reported.

Methods.—Seeds of the following commercial varieties of *Ricinus communis* were grown in pots: Zanzibarensis; Borboniensis Arboreus; Red Spire. Material was obtained from plants of varying ages, using both seedlings consisting of a few internodes as well as older plants of many internodes and considerable secondary growth. Both the apical regions and older portions of the stem were investigated. The material was either killed in CRAF or in a mixture of equal parts of two per cent acetic acid and one per cent osmic acid.

In addition a considerable amount of fresh material was sectioned by hand, and the effects of various microchemical reagents, especially of osmic acid, were directly studied under the microscope. The material killed in CRAF was stained with Safranin and Fast Green; the material fixed in the osmic-acetic acid mixture was not stained any further or was slightly counterstained with Fast Green only.

Results

1. *First appearance of secretory cells.*—The general distribution of the cells in definite patterns was confirmed, though there occurred slight qualitative and quantitative differences between the individual plants. The cells make their first appearance very early within the terminal bud of the stem and in the primordia of the leaves (Figs. 1, 8). Very small secretory cells which already have undergone some differentiation may be recognized within the small-celled outermost region of the meristematic corpus with ordinary staining methods. These cells show contents more highly refractive than the rest of the ground parenchyma cells; they occur both in the youngest internodal regions, and, somewhat more conspicuously, in the nodal diaphragms.

How near the apex this first *visible* differentiation occurs, is subject to certain variations. Both in the Zanzibarensis and Red Spire varieties terminal buds were found in which the first visible differentiation occurred in practically the youngest zone of the corpus, while in others it occurred in somewhat more mature regions of the apical meristem.

The further development and distribution of secretory cells is largely determined by the pattern of differential meristematic activity in the stem. Cells in the nodal regions undergo comparatively few divisions, and here the secretory cells appear from the beginning more crowded and in greater number. In the typical internodal rib meristem, on the other hand, the pattern of cells is worked out more gradually. In the peripheral zone of the internode, for example, near the bases of the leaves, groups of secretory cells may be seen in longitudinal section which are typical for this position. They are visible very early near the apex, but occasionally their appearance becomes somewhat delayed though finally they will always differentiate in the same position.

Similarly, the pattern of distribution of secretory cells in the epi-

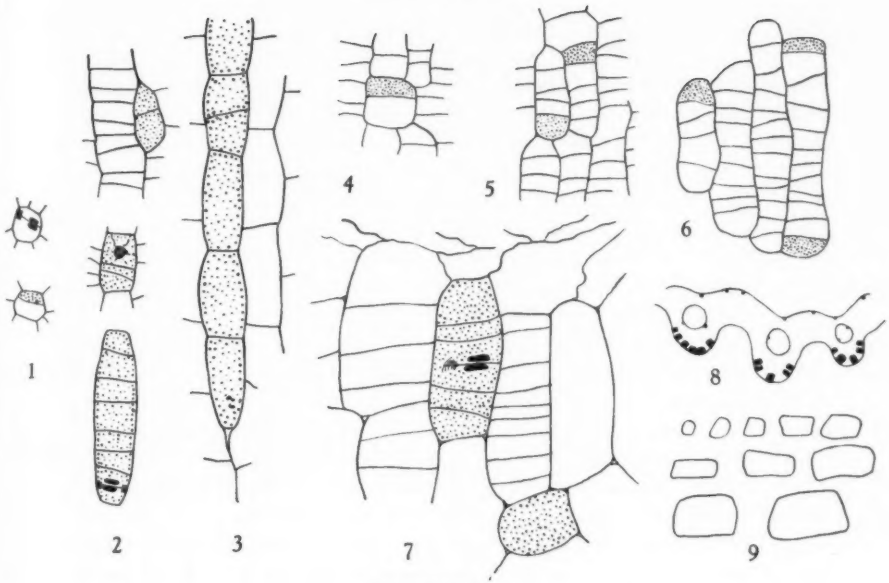
dermis of the young leaves is determined and becomes visible, when the leaf is still in its primordial stage. The position of the secretory cells opposite the veins is here very characteristic and gives excellent evidence for the early blocking out of particular cell patterns (Fig. 8). It is known that certain kinds of specific cells are differentiated relatively early, for example, procambial cells, stoma cells, cells containing raphids of calcium oxalate, and the hydathode cells in the leaves of *Lobelia* recently reported by Philipson (8).

2. *Formation of cell files and of solitary secretory cells "de novo" in older regions.*—The secretory cells which are located within the intercalary rib meristem of the internodal regions continue to divide along with the rest of the cells of the ground parenchyma. Thus gradually very long files or rows of secretory cells are formed which eventually consist of many elements (Figs. 2, 3).[1] Sometimes periclinal divisions occur, resulting in double rows. Occasionally in these files the nuclear division figures can be observed (Fig. 2), and since the files are always continuous, there is good evidence that no secondary reversion to parenchymatous character of the secretory cells takes place while dividing. Secretory cells in the nodal regions, on the other hand, in conformity with the rest of the cells there, rarely divide.

In older internodes, the intercalary meristems of which are still very actively dividing, small solitary secretory cells can generally be seen (Figs. 4, 6). Obviously these stem from recent divisions since they could not have been maintained from the apical region in this condition without quite considerable increase in length and ensuing divisions. Therefore, in addition to the formation of secretory cells in the apical meristem, cells are also formed *de novo* in the larger (much more vacuolate) cells of the more mature regions. These later formed cells either remain undivided or give rise to correspondingly shorter files of secretory cells. As indicated above, the appearance *de novo* of secretory cells can often be observed in the regions at the bases of the leaves.

An interesting correlation, however, was found to exist between the degree of proximity of secretory cells to the apex and the tendency to form solitary cells *de novo* in older internodes. It was noted that

[1]These rows are a useful tool for the determination of the number of cell generations which are derived from one primordial cell.



FIGURES 1-3

Development of rows of secretory cells in young and older internodes. Longitudinal sections. The stem apex is toward the top of the page. x 175.

FIGURE 1

Small meristem cells from the second internode of the terminal bud, showing unequational divisions and first appearance of secretory cells.

FIGURE 2

Development of rows of secretory cells. Two-cell-stage above; nuclear division figures in center and below.

FIGURE 3

Lower part of a row of mature secretory cells from old internode.

FIGURES 4-6

Formation of secretory cells *"de novo"* in the internodal meristems of older portions of the stem. Longitudinal sections. x 175.

FIGURE 4

Large ground parenchyma cell from which a secretory cell has been cut off by an unequational division.

FIGURE 5

Secretory cells at the ends of two rows of cells, each of which has been derived from one parenchyma cell.

FIGURE 6

Four similar, but longer rows.

FIGURE 7

Cross section through pith of old internode, showing meristematic activity induced by wounding; in the center row of secretory cells has been derived from one original secretory cell. The wound surface is toward the top of the page. x 175.

FIGURE 8

Cross section through very young leaf in terminal bud, showing the distribution and early differentiation of secretory cells within the embryonic tissue, particularly in the leaf epidermis opposite the procambial strands. x 75.

FIGURE 9

Range of size of secretory cells formed in different parts of the stem, as seen in longitudinal sections of the youngest and older zones. x 175.

the latter were formed particularly in the stems of those plants in which the first visible differentiation of the cells took place at a somewhat greater distance from the apex. On the other hand, very early determination and visibility of the specialized cells at the apex was correlated with a somewhat smaller tendency to form solitary secretory cells in the ground tissue of the older internodes.

3. *The method of formation of the secretory cells.*—The first step in the analysis of the specific development of a cell in the midst of more or less identical ground tissue elements must obviously be the observation of any peculiarities in its morphological development and intercellular relationships. In the present case, there is some evidence that the division which results in the subsequent formation of the secretory cell is unequational and in some way differential. Numerous cases were seen in which a relatively small secretory cell was located adjacent to a considerably larger parenchymatous cell from which it had been cut off by a recent division (Figs. 1, 4). It is not easy to discover such relationships in zones of the intercalary meristem in which considerable longitudinal stretching and division of the cells have already taken place since the mutual adjustment of the cells tends to obscure the original relationships. However, it is frequently possible to recognize entire files of secretory cells whose general outline indicates that they have been derived from one original cell, and similarly there are cases where derivatives can be distinguished from an originally single parenchymatous cell in which solitary secretory cells have been formed *de novo*. In these cases it was noted that not infrequently the secretory cell was one of the "end cells" in such a parenchymatous file (Figs. 5, 6). However, this is not always the case since in other instances secretory cells occurred elsewhere in the rows.

Since in *Ricinus* the divisions which lead to the formation of the secretory cells occur over a considerable period during the development of the plant (Fig. 9), it must be concluded that the differences of the mother cells in size and in degree of vacuolation, which are considerable, have little influence on their capacity to divide differentially. Differential divisions in other cases, such as root hair cell divisions or stoma cell divisions do not show this latitude, the critical divisions as a rule occurring at a rather more definite size of the mother cell. The secretory cells themselves, as mentioned

previously, retain the capacity of division. This appears the more remarkable as their contents, to judge from the microchemical reactions, undergo changes of various kinds. This becomes at once evident when comparing the appearance of the cells in zones of the meristem with that in fully maturated regions of the stem. In the variety Red Spire the secretory cells contain a natural red pigment which increases in depth of coloration with the age of the cell and makes it possible to locate the secretory cells in the tissues without the help of reagents. But even in the actively dividing younger regions of the stem there can generally be detected finer differences in the properties of the contents of the cells with the help of microchemical agents, especially osmic acid. If sections taken from fresh materials are treated with this reagent and the reaction which takes place gradually is followed under the microscope, finer differences in the coloration of cells can be seen everywhere in the young tissues; one observes, for instance, that in dividing rows of secretory cells the more recently formed cells stain a much lighter blue than the rest.

The various observations here recorded must, however, be regarded as exploratory in nature and it would be desirable to attempt finer analyses of the changes in both nucleus and cytoplasm before, during and after division, in regions where the specific cells arise. The problem of differential cell divisions is probably to a large degree synonymous with or related to the more general one of polar, unequational cell division which involves unequal distribution or segregation of nuclear and (or) cytoplasmic material and the conditions which provoke these. Tests for intracellular changes, such as may be carried out with nucleic acid, may be applied to cases like the present one.

4. *The relation of the differentiation of secretory cells to their position in the tissue.*—The distribution of the secretory cells occurs in regular patterns in very different locations in the plant. It appears, therefore, unlikely that relatively simple external conditions, such as are known to be effective in the differentiation of suberized cork cells or lignified hypodermal brachysclereids near a surface (Bloch, *2*), could be the determining factor in their formation. The cells are formed both in embryonic and in older regions, in the interior and at the surface, within the ground tissue and along vascular strands.

5. *Experiments to change the character of the secretory cells.*—

One of the main supports for the theory that the external conditions of the environment are the major inducing agents of cellular differences in plants has been the fact of experimental dedifferentiation and redifferentiation (Bloch, 1). It has been shown, in experiments concerned with wound healing and regeneration, that the cells of multicellular plants, both in normal and regenerative ontogeny, remain potentially pluripotent. This applies particularly to the parenchymatous and collenchymatous elements of the ground tissue the walls of which remain for a long time in a plastic condition. On the other hand, there are many cells whose contents and walls become specialized, such as the elements of the xylem and phloem, crystal cells, sclerenchyma cells and others. These cells generally react to the regenerative stimulus only in their early developmental stages. They either soon lose the power of division, or, in heavily incrusted cells of the sclerenchyma, the power of growth. In plants one of the main impediments to renewed growth of cells is the condition of the differentiated cell wall which in many ways lends to the cells their specific morphological character. In animals, it is known, the situation is different in that many cells, though acquiring their specific character early, keep on dividing and producing their own specific kind, even in non-specific environments such as tissue cultures.

The remarkable fact that the secretory cells of *Ricinus* continue to multiply and "breed true," like some animal cells, suggests that the changes which take place subsequent to their first differentiation early in the apical meristem have little effect on the factors which control the power of mitotic division and on those which control the growth of the cell wall. In order to test this, young and some old internodes were wounded. It was found that, both in the region where the internodal meristems were still active and in those where activity had long ceased, new wound meristems were formed in the ground tissue of the cortex and pith. The secretory cells divided vigorously in the same way as the cells of the ground parenchyma, but gave rise only to secretory cells. Thus, neither renewed cell division and growth nor the changed position below the surface of the wound meristem resulted in any alteration of their previously acquired anatomical and physiological character (Fig. 7).

These observations would seem to indicate that in previous studies botanists may not have sufficiently taken account of the possibility

that in the somatic differentiation of multicellular plants certain cells may become determined and stabilized in their character very early and subsequently give rise to cells of the same character only, even under conditions which ordinarily induce dedifferentiation. The majority of potentially meristematic plant cells do not react in this manner. They remain, if they stay reactive at all, in a more or less undetermined state in which their development can be tipped one way or another under the selective control of the environment.

Further experiments are required to obtain additional evidence as to the nature of the stabilized change which has taken place. Whether this change is of a truly irreversible kind could be tested by devising proper methods of growing the cell in culture. Preliminary experiments were made to grow internodal tissues in tissue culture, using White's[2] medium either pure or with addition of napthalene acetic acid in concentrations of 10^{-6} and 10^{-8} gm. per ml. (see 14, page 103). Though it was possible to obtain sterile cultures of both apices and older internodal regions, and to maintain the cultures through 6 passages during a period of 6 months, only limited growth was obtained, and the results cannot yet be considered conclusive. However, there was no indication of a dedifferentiation of the secretory cells which were present at the time of start of the culture. In the early stages of the cultures the parenchymatous ground tissue at the surface of the excised piece proliferated very considerably, the hypertrophying cells forming a loose mass of intumescence-like elements. Although during this process the secretory cells in this mass reached many times their original size and divided also, their peculiar contents could still be demonstrated since osmic acid produced some coloration in them. In the later stages of callus formation, however, the general differentiation in the callus was so low that it was difficult to determine whether new secretory cells had been differentiated at all. It thus remains to be determined whether under suitable conditions such cells will be produced in culture and be brought to maintain themselves.

6. *Summary and Conclusions.*—The secretory cells of *Ricinus communis* are cells of an idioblastic character which combine the following features: (1) they are formed in definite distribution and

[2]The writer is indebted to Dr. White for his valuable advice and assistance in the preparation of the cultures.

patterns occurring throughout the plant body in specific positions, both in the primary and secondary tissues; (2) they appear as very early determined cells in the primordial meristematic regions of the apex, and are, on the other hand, also formed *de novo* in the ground tissue of older internodes; (3) they frequently arise from unequational, probably differential divisions of cells of the ground tissue; (4) they retain, in spite of their specialized character, to a remarkable degree the power of multiplication, producing either cell rows of considerable length or exhibiting the capacity of renewed division and growth in artificially induced wound meristems in older regions. They do not, however, revert to a non-specific parenchymatous condition, as known for many other plant cells.

The secretory cells are remarkable because they arise under great latitude of external conditions and morphological positions in the plant, and because subsequently they combine physiological specificity with a definite potentially meristematic character. We are used to associate in plants the concept of a differentiated cell with changes in its cytoplasmic phase and especially in the cell wall which as a rule, becomes an impediment to its further growth and multiplication. The present case does not wholly agree with this concept, and the behavior comes to mind of certain cells in the tissues of animals which acquire their morphogenetic character early in ontogeny and maintain it in specific cell lineages during normal ontogeny as well as in non-specific environments through mitotic divisions.

The writer has previously suggested a possible mechanism (Bloch, 4), which might induce the individual character in cells such as described here. It was concluded that, if no genetic change of a nuclear nature were involved, a specific stabilized alteration must have taken place in the reactivity systems of their cytoplasm. This change, it was suggested, might occur as the result of a differential, polar division. At the cell division which precedes the formation of a secretory cell, an unequal distribution or qualitative segregation of cytoplasmic material might take place (for possible segregating mechanism cf. Costello, 5), resulting in differential concentration of determinants or determinant-precursors in the two daughter cells. To account for the stabilized change in the cells, the result must involve specific, qualitative alterations in the reactivity systems of the cells. These alterations are associated with changes in the cytoplasm

which may be called genetic in nature in that they are responsible for the evident transmission of cellular specificity along somatic cell lineages.

The problem of cellular specificity and individuality is of supreme interest to biologists as a whole since it involves both the problems of normal and pathological development. To the writer it would appear that material as described here might be particularly favorable for the analysis of conditions which bring about such pronounced cellular individuality. Although the formation of the secretory cells constitutes a part of the normal tissue differentiation in *Ricinus,* their specific and individualistic character, not readily influenced by changes in the environment, reminds us of those pathological cells in animals which develop individual and detrimental properties secondarily and which cannot be controlled by the organism as a whole. Attempts have not been lacking to obtain evidence from plant material as to the causes of atypical, malignant growth in which there occurs such a loss of morphogenetic control. The efforts, however, have been concentrated so far entirely on pathological forms of plant growth, namely the well-known, more or less unorganized structures of bacterial galls, especially crown gall (E. F. Smith and others, cf. Riker et al., 9), and more recently, on the secondary derivatives of such galls for which at present no inducing agent is known (cf. White and Braun, 15). The writer, in conformity with others, believes that a more thorough study of the processes of normal differentiation constitutes a first essential toward the solution also of this important and urgent problem in biology.

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THE GROWTH OF THE DIGESTIVE SYSTEM IN THE FETAL DOG

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Very little of the literature on the digestive system concerns its quantitative development or the quantitative analysis of the adult system. A few of the papers of this nature are those by Kearney (1914) on the embryonic and young dogfish, Lowrey (1911) on the prenatal growth of the pig and the work of Latimer (1934a and 1934b) on the growth of the fetal cat. The quantitative growth of the human fetus has been studied by Jackson (1909), Scammon and Kittelson (1924) and the human pancreas by Scammon (1924).

This paper will present the increase in weight of the digestive system and its parts; the digestive tube, the liver and the pancreas. The growth of the four parts of the digestive tube, the esophagus, stomach, small and large intestine, will also be shown. All of these will be shown plotted on body weight and also on body length. The relative weights will also be given. The linear dimensions are not given. These puppies were preserved in the same manner as the fetal cats but the intestines of these puppies were so brittle when they were straightened out that it was impossible to make satisfactory measurements of the lengths of the intestines. The small intestines of the dog fetuses seemed to be more tightly coiled also than were those of the fetal cats. Aside from this there seems to be no satisfactory explanation why the intestines of these dogs could not be straightened out sufficiently for measuring as were the cat intestines.

MATERIALS AND METHODS

The digestive systems of 140 fetal and newborn puppies were studied. Of the 140 specimens, 24 were newborn puppies. All of these were born during the night and brought to the laboratory the first thing in the morning, chloroformed and then prepared in the same manner as the fetuses. They were very nearly divided as to sex as

there were 70 males, 66 females and four of the smallest were too small to determine the sex without a microscopic examination. These 140 specimens represent 30 litters, having from one to nine per litter. They ranged in weight from 3 to 493 grams, and from 55 to 257 millimeters in body length (nose-anus).

Each pup was embalmed by the injection of ten per cent formalin solution into the thoracic cavities and into the abdominal cavity and the top of the skull was opened so that the solution could enter and preserve the brain and spinal cord. The fetuses were then stored in an ample amount of a ten per cent formalin solution for at least three months so that they might be uniformly and well preserved. Each fetus was tagged, of course.

After adequate preservation each fetus was removed from the solution, all excess formalin solution was removed with paper toweling and then the entire fetus was weighed, to a tenth of a gram on a laboratory balance for the larger specimens and the smallest specimens, to the nearest milligram in a glass stoppered weighing bottle on a chemical balance sensitive to a tenth of a milligram. All of the viscera were carefully dissected out and placed in a moist chamber until they were transferred to glass stoppered weighing bottles and weighed on the chemical balance to a milligram for the larger parts and to a tenth of a milligram for the smaller parts. The other organs and systems will be reported later and only the digestive system will be discussed in this paper.

After the removal of the thoracic and cervical organs, with the exception of the esophagus, the abdominal cavity was opened and the liver was first carefully removed with all vessels and mesenteries cut close to the liver. The pancreas was then removed, separating it from the adjacent fat in the older specimens. In the smaller specimens, with little fat developed, this was an easier and also a more accurate dissection than it was in the older fetuses and newborn puppies. The diaphragm was then split and the esophagus was freed from the adjacent tissues and cut at its entrance into the stomach. The cardiac sphincter made an excellent landmark for this incision. The upper end of the esophagus was cut just inferior to the pharynx. Next the large intestine, including the caecum, was removed. It was divided as it entered the skin of the anal region and the union of ileum and colon was an easily found landmark. Then

the small intestine was carefully freed of its mesentery and each part of the digestive tube, together with the liver and pancreas, stored in a moist chamber until weighed. All fat and mesentries were removed before weighing each organ. With increasing size of the fetuses there was an increasing amount of meconium in the large intestine. This and the amnionic fluid found in the stomachs of the smaller fetuses and the milk found in the stomachs of some of the newborn pups were removed. The meconium was carefully "milked" out of the large intestine and also, in a few of the older fetuses, from the terminal part of the ileum. The gastric contents were removed and the stomach washed out through a longitudinal incision made along the greater curvature of the stomach.

No tables of weights are given, but the formulae are given for each organ for both body weight and body length. The curvilinear formulae were developed by trial and error and the rectilinear formulae were developed by the method of least squares (Snedecor, 1938). The weights falling in each ten grams increase in body weight or each 20 millimeters increase in body length were averaged and the central tendencies of each group were used in plotting the curves rather than the entire 140 weights. The formulae should be used only within the limits indicated for each formula.

The weights of each organ were reduced to percentages of the total body weight and these percentages were plotted on preliminary graphs, against both body weight and body length, the same as for the absolute weights. Curves were drawn on the preliminary chart for each percentage. Formulae for these curves were developed by the method of least squares for all of these percentage curves except two which were too irregular to be fitted easily. These two curves were drawn in by inspection, fitting the cases as well as possible. These lines without the individual cases are shown in the figures.

We wish to express our most hearty thanks to the Physiology Department for permitting us to get these fetuses. The fetuses were taken from dogs being used for short experiments. None of the mother dogs were used in prolonged experiments lasting over several days. In all cases the fetuses were taken within three or four hours after the start of the experiment.

Digestive System

The weight of the entire digestive system plotted on body weight is shown in Figure 1. Both weights are in grams. The weights of the males are indicated by dots and the females, by circles. The line representing the growth of the digestive system was drawn from the formula:

$$Y = 0.112X - 0.44,$$

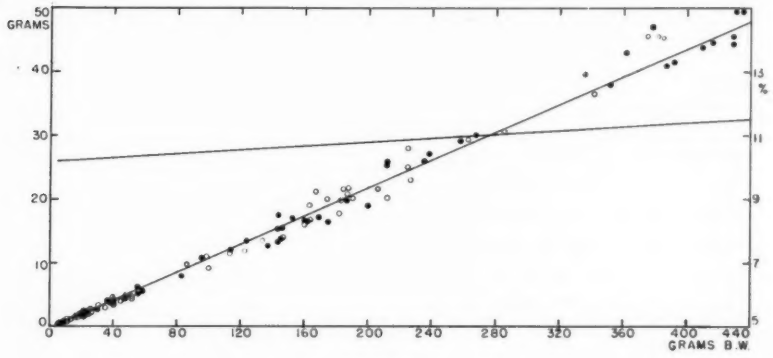

FIGURE 1

The weight of the digestive system in grams, plotted on body weight, also in grams, is shown in the rectilinear curve with the individual cases shown as dots for the males and circles for the females. The straight line without any cases represents the percentage increase of the digestive system. The percentage values are given at the right and the weights in grams at the left. Formulae from which both curves were drawn are given in the text.

from 10 to 440 grams of body weight. The average difference between the calculated and the observed values was 8.12 per cent. The weights of the newborn digestive systems were well scattered among those of the fetal dogs and so they are not indicated separately in this figure. Of course most of them came in the upper range of the body weights.

The lighter line without any cases represents the weights of the digestive system expressed as percentages of the body weight. The percentage values are given in the right margin. The cases were more scattered, especially in the smaller fetuses, than are the individual weights shown in the figure showing the growth in weight. The formula for this line is,

$$Y = 0.003X + 10.15.$$

This line seemed to fit the averages better than that for the weight in grams, for the average deviation of the calculated from the observed averages is 5.59 per cent.

The weights of the entire digestive system in grams plotted on body length measured in millimeters are shown in Figure 2. As in

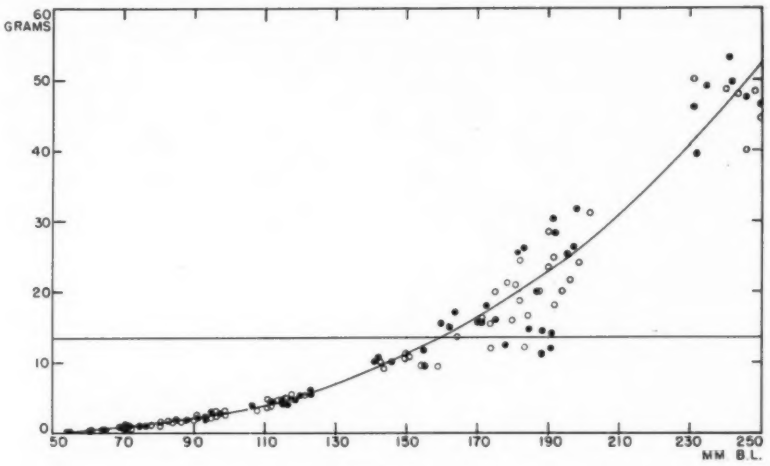

FIGURE 2

The growth of the digestive system plotted against body length in millimeters is shown in the curve concave superiorly and with the dots representing males and circles, females. The horizontal line represents the percentage weight of the digestive system. It averages 10.35 per cent of the body weight. The formulae used in drawing the curve representing the increase in grams are given in the text. The figures in the left margin represent both weights in grams and percentages.

the preceding figure, the dots represent males and the circles, females. The numbers at the left represent grams, or percentages for the percentage curve. The line concave superiorly with the cases representing the growth of the entire digestive system was drawn from the formula:

$$Y = \frac{(0.1X)^{3.6}}{1607}, \text{ from 55 to 157 mm. of body length and,}$$

$$Y = \frac{(0.1X)^{3}}{308}, \text{ from 157 to 250 mm. of body length.}$$

The average percentage deviation of the calculated values from the observed values is 4.93 per cent, or this curve fits the cases a little better than the straight line curve plotted on body weight and shown in Figure 1. The percentage weights of the entire digestive system were plotted on body length as well as on body weight and the horizontal line in this figure represents the average of 10.35 per cent. These percentages were tested and there was no significant difference in the fetuses of various body lengths but merely individual variations from this average.

Thus we see that the weight of the entire digestive system, including the digestive tube, the liver and the pancreas forms an arithmetical function of the body weight throughout the entire fetal period and it increases more rapidly, as shown by the curve convex superiorly in Figure 2, than the body length. The percentage weight remains constant with reference to the body length but it increases slightly in the heavier fetuses and in the newborn puppies.

There are very few data on the weights of the entire digestive system. Jackson (1909) gives the percentage weights of the liver, pancreas, stomach and intestines and the sum of these weights has been determined for each month from the second fetal month to birth. In a few of the periods the weights of one or more of the parts are not given but the average of the sums of these parts as far as they are given is 7.3 per cent of the body weight, or the digestive system of the dog is nearly 42 per cent heavier than that of the human fetus. Jackson's figures do not include the esophagus, but it forms only a small percentage of the total digestive tract in the dog fetus.

The digestive tube, the liver and the pancreas were reduced to percentages of the total digestive system and these percentages are shown graphically in Figure 3. The digestive tube and the pancreas increase in their percentages, and the liver decreases during the fetal growth. The digestive tube, or the esophagus, stomach, small and large intestines increases from a little over 14 per cent of the entire digestive system in the smallest fetuses to about 29 per cent at 160 grams of body weight and thereafter its rate of increase is much slower, increasing to about 33 per cent in the largest fetuses and newborn dogs. The maximum percentage of the liver, or 85 per cent, is found in the smallest fetuses. This decreases to about 70 per cent at 160 grams of body weight and then more slowly to about 65 per

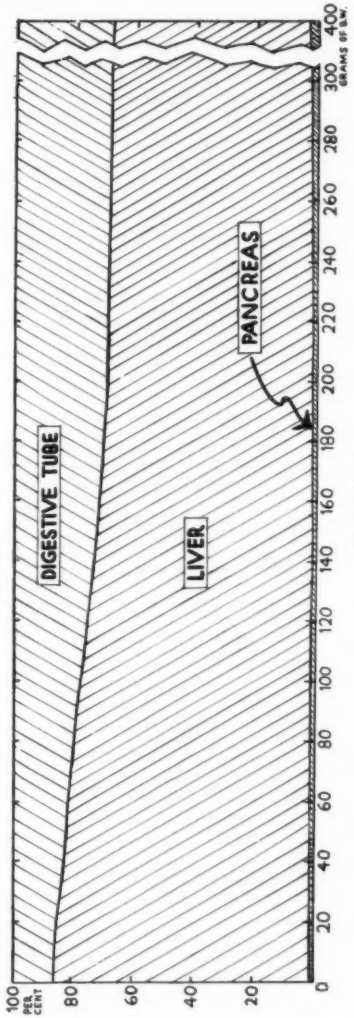

FIGURE 3

Graph showing the percentage changes in the parts of the digestive system.

cent in the largest specimens. The pancreas increases irregularly, from a minimum of about 0.6 per cent in the smallest specimens to about 2.5 per cent in the largest specimens. The decrease in the percentages of the liver and the increase in relative weights of the pancreas and the digestive tube in the dog is very similar to that found in the fetal cat (Latimer, 1934b).

Liver

The liver has been shown (Fig. 3) to be the largest division of the digestive system and its growth in weight plotted against body weight is shown in Figure 4. As in the preceding figures the dots represent the weights of male livers and the circles, female. The line representing the weight of the liver plotted on body weight, with all the weights in grams was drawn from the formula:

$$Y = 0.072X + 0.45.$$

This formula is valid between the body weights of 10 and 410 grams of body weight. The average percentage deviation of the calculated from the observed weights is 5.08 per cent. The figures in the left margin represent weights in grams for this curve.

The line without any cases and decreasing from about 7.6 per cent in the smallest specimens to a little less than 6 per cent in the largest,

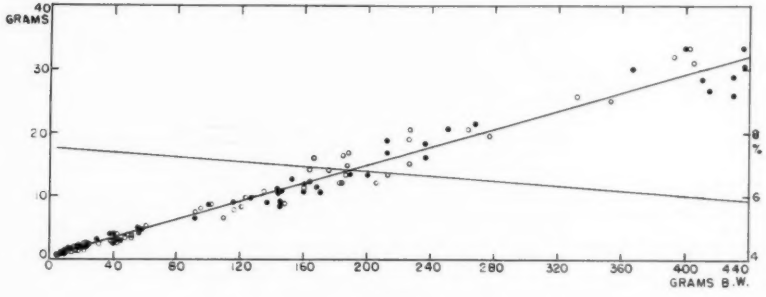

FIGURE 4

The weight of the liver measured in grams and plotted against body weight. The dots represent males and the circles, females. The straight line decreasing from left to right and without any cases represents the percentages of the liver. The percentage values are given at the right and the weights in grams at the left. The formulae used in drawing both of these curves are given in the text.

represents the weights of the liver as percentages of the body weight and plotted on body weight. As in the preceding figures, the individual percentages were plotted and the curve was fitted by means of least squares and the line only was drawn in this figure. The line was drawn from the formula:

$$Y = 7.60 - 0.004X.$$

There is a good deal of variation in the percentage weights of the liver, the average deviation of the calculated from the observed values in this curve is 11.50 per cent. This is slightly over the ten per cent value which indicates a good fit for a curve. This large percentage deviation is due to the scatter of the individual cases as shown on the preliminary graph, or the variation in the percentage weight of the individual livers. The percentages in this figure are shown in the right margin.

The same weights of the livers plotted on body length measured in millimeters is shown in Figure 5. Again the dots represent males and the circles, females. This curve was drawn from the empirical formula:

$$Y = \frac{(0.1X)^{2.8}}{256} - 0.25.$$

This formula is valid between the body lengths of 55 and 255 mm. This curve is not quite as well fitted to the cases as the growth curve shown in the preceding figure, for the average deviation of the calculated from the observed values is 5.82 per cent. This is still a good fit however. The weights of the liver in grams are shown in the left margin.

The lighter line without any cases, represents the percentage weights of the liver plotted on body length. The cases were plotted as in the preceding figures and only the line determined by the method of least squares is shown in this figure. The figures in the left margin, represent not only the weights in grams but as well the percentages of the liver weight. The line showing the percentages of the liver on body length was drawn from the formula:

$$Y = 8.7531 - 0.006X.$$

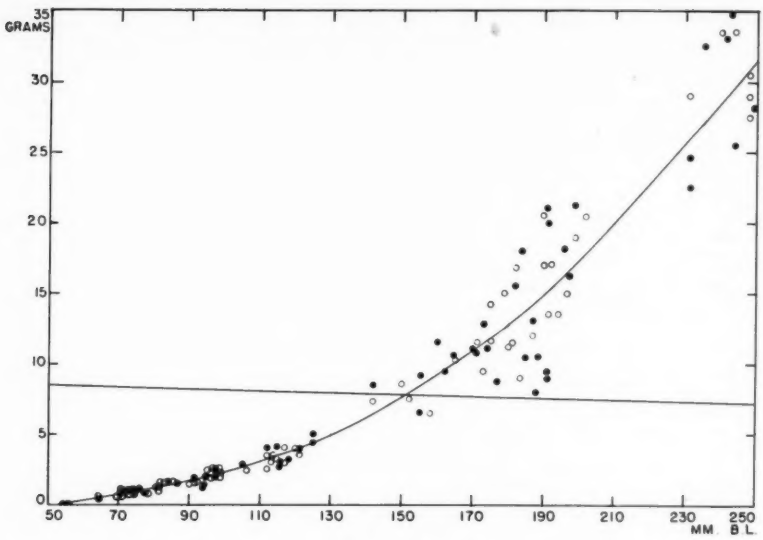

FIGURE 5

The weight of the liver plotted on body length is shown in the curve concave superiorly and with the dots representing males and the circles, females. The straight line without any cases represents the percentage values of the liver. Formulae for both curves are given in the text. The figures in the left margin give both weights in grams and percentages.

The average percentage deviation of the calculated from the observed percentage values for this curve is 5.24 per cent.

In general, the liver is heaviest in the smallest specimens, decreasing regularly with reference to both body weight and body length. The weight of the liver in grams, increases as an arithmetical function of the total body weight and when plotted on body length, the liver weights form a curve concave superiorly, or the liver weights increase as an algebraic function of the body length.

The cat liver (Latimer, 1934b) shows growth curves similar to those shown in Figures 4 and 5 except that the curves are not quite so regular. The percentage curves of the cat liver show a more definite maximum early in the fetal period than is shown for the dog liver. There is a slight maximum for the dog liver but the straight line seemed to give a more satisfactory curve than an irregular curve

showing this very slight initial maximum. The liver in the fetal dog seems to be slightly heavier than that of the fetal cat. The dog liver averages higher than the cat liver at the beginning of the fetal period, and the individual cases (maximum cases) are about 9 per cent in the cat and 11.5 per cent in the dog. The average for the newborn dogs is about 6 per cent and for the cats about 4 per cent. The data given by Jackson (1909) and Patton (1946) for the fetal human liver resemble that for the dog in that they decrease from an early maximum, but the human liver seems to be somewhat lighter in relative weight than the fetal dog liver.

Pancreas

The pancreas, the smallest division of the digestive system, is shown plotted against body weight in Figure 6. The weights of the pancreas in grams are shown in the left margin and the body weights, also in grams, as the abscissae. The pancreases of the males are shown as dots and those of the females as circles. The line representing the growth in weight of the pancreas from 6 to 155 grams of body weight was drawn from the formula:

$$Y = \frac{X^{1.41}}{4620}, \text{ and from 155 to 440 grams,}$$

$$Y = 0.0034X - 0.28.$$

The average deviation of the calculated from the observed weights was 11.68 per cent, or the greatest average deviation of any of the curves in this study. The pancreas is a very small organ in the smaller fetuses and it is difficult to remove accurately as it is not encapsulated. In the smallest fetuses there was no fat adjacent to the pancreas, but in the larger specimens it was difficult to separate the pancreas from the fat.

The line without any cases and rising irregularly from 0.05 per cent to a maximum of 0.28 per cent represents the percentage weight of the pancreas. No attempt was made to develop a formula for this curve for its percentage changes were too irregular. There seem to be two periods of more rapid growth, the first from 6 to 40 grams of body weight and the second from 120 to 170 grams of body weight.

The weights of the pancreas plotted on body length in millimeters

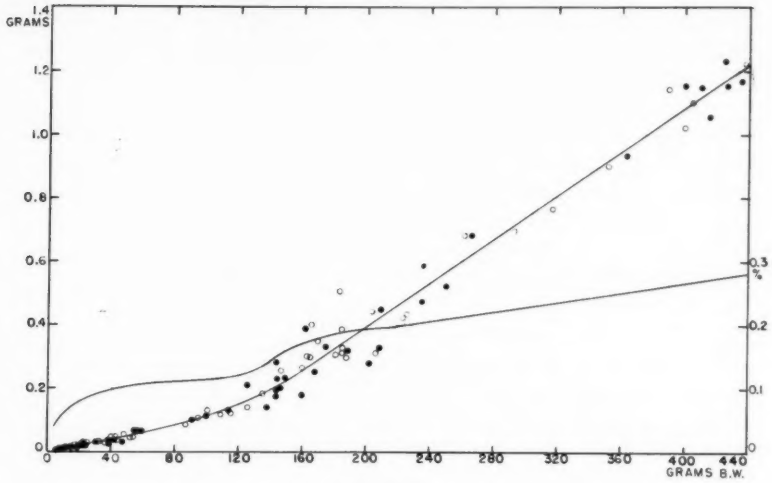

FIGURE 6

The weight of the pancreas plotted on body weight is shown in the curve concave superiorly at first and then forming a straight line. The formulae used in drawing this curve are given in the text. The line rising irregularly at first and then becoming straight and without any cases represents the percentages of the pancreas.

are shown in Figure 7. The dots and circles represent males and females, as before. This curve was drawn from the formula:

$$Y = \frac{(0.1X)^{4.2}}{591528} - 0.0012, \text{ from 60 to 255 mm. of body length.}$$

The average deviation of the calculated from the observed values for this curve is 7.35 per cent. The straight line rising from 0.04 per cent at 65 mm. of body length to 0.26 per cent at 250 mm. of body length represents the percentage weight of the pancreas. This line was drawn from the formula:

$$Y = 0.0012X - 0.0366.$$

The average deviation of the calculated from the observed percentages is 7.51 per cent.

The two figures show that the growth of the pancreas is slow in the early part of the fetal period, and that it accelerates in its growth

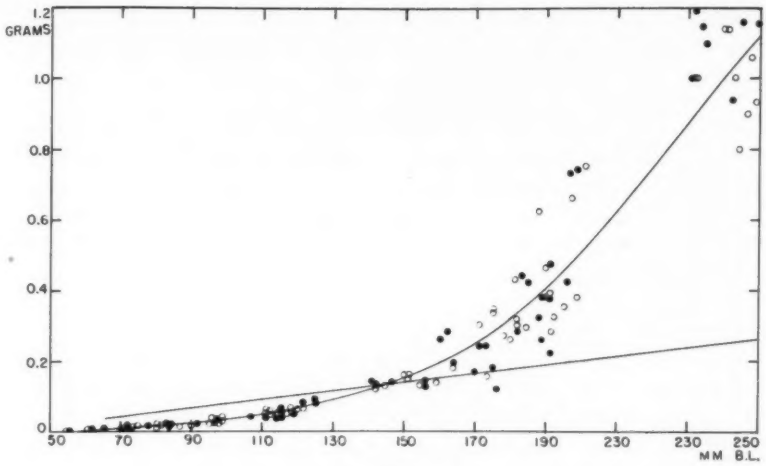

FIGURE 7

The curve concave superiorly and with the dots representing males and the circles, females shows the increase in weight of the pancreas plotted on body length. The straight line without any cases represents the percentages of the pancreas. Formulae for both curves are given in the text. The figures in the left margin represent both grams and percentages.

rate in the later part. This is true when the weights of the pancreas are plotted on either body weight or body length. The percentage weight of the pancreas increases throughout the fetal period, or from 0.04 to 0.26 per cent of the body weight. The relative growth of the pancreas in the fetal cat resembles that of the fetal dog except that there is a greater range in the percentages in the dog. The weights of the pancreas relative to the body weights seem to vary a good deal in the different animals studied. Jackson (1909) finds the pancreas in the human fetal period increasing from 0.032 to 0.145 per cent, Patton (1946) reports percentage weights of the pancreas in man of much lower values, or increasing from 0.01 to 0.10 per cent of the body weight. Lowrey (1911) gives a percentage value of 0.16 per cent for the weight of the pancreas in the newborn pig. In all of these forms the pancreas is small at first and increases more in the later part of the fetal period. Scammon (1926) shows the weights of the fetal human pancreas increasing as an arithmetical

function of the body weight and as a power curve when the pancreas weights are plotted on body length.

Digestive Tube

The weight of the digestive tube, or the weight of the esophagus, stomach, small and large intestines is shown plotted on body weight in Figure 8. The weights of the individual digestive tubes are shown in grams in the left margin and the body weights in grams as the abscissae. The curve from 6 to 190 grams of body weight was drawn from the formula:

$Y = (0.01X)^2 + 0.013X$, and from 190 to 440 grams of body weight, $Y = 0.039X - 1.33$.

The average deviation of the calculated from the observed averages for these two formulae is 5.01 per cent.

The line rising irregularly from 6 to 200 grams and without any cases shown represents the percentage weights of the digestive tube. From 200 to 440 grams of body weight the percentage increases as an arithmetical function of the body weight. The percentage values are given in the right margin.

The same weights of the digestive tube plotted on body length are shown in Figure 9. The contents of each digestive tube were

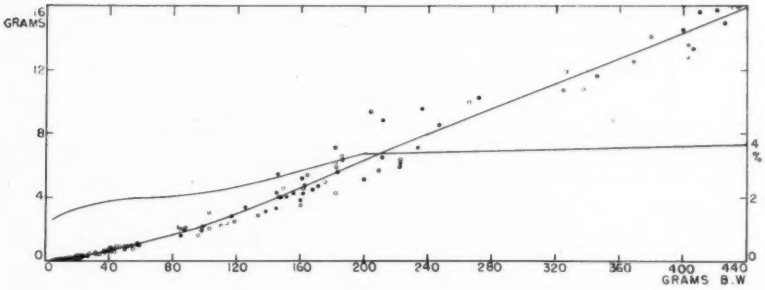

FIGURE 8

The weights of the digestive tube are represented by the curve concave superiorly at first and then a straight line. The individual cases are shown as dots for the males and circles for the females. The formulae used in drawing this curve are given in the text. The line without any cases and rising irregularly at first and then forming a straight line represents the percentages of the digestive tube.

removed before weighing and the weight of the digestive tube as here given is really the sum of the weights of the esophagus, stomach, small and large intestines. From 55 to 143 millimeters of body length, this curve was drawn from the formula:

$$Y = \frac{(0.1X)^{4.6}}{93185} + 0.0057, \text{ and from 143 to 260 mm.,}$$

$$Y = \frac{(0.1X)^{3.6}}{6497}$$

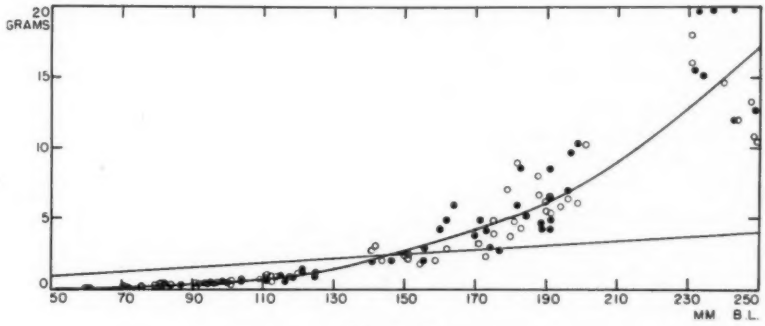

FIGURE 9

The growth of the digestive tube plotted on body length is shown in the curve concave superiorly, with the dots representing the males and the circles, the females. The rectilinear curve represents the percentages of the pancreas. Formulae for both curves are given in the text. The figures in the left margin represent both grams and percentages.

The average deviation of the calculated from the observed averages for the first curve is 1.41 per cent and for the second, 1.19, or an average of 1.31 per cent for the entire curve. The straight line without any cases and which rises slowly represents the percentages of the digestive tube. The numbers in the left margin marked grams show the weights in grams and they also serve to show the percentages of this percentage curve. This line was drawn from the formula:

$$Y = 0.0144X + 0.30, \text{ from 55 to 250 mm. of body length.}$$

The average deviation of the calculated from the observed percentages is 3.74 per cent.

There are very few data on the growth of the digestive tube. Jackson (1909) gives the weights of the stomach and the intestines and the sum of these seems to vary a good deal, with the maximum percentage of 2.65 per cent in the second fetal month. The average of these percentages of the human fetal digestive tract, but not including the esophagus, is 1.55 per cent. Lowrey (1911) finds that the stomach and intestines increase gradually throughout the fetal period and near term average 3.6 per cent of the body weight. Thus the digestive tube of the fetal pig resembles the growth in weight of the digestive tube of the fetal dog rather closely.

The weights of the four parts of the digestive tube, or the esophagus, stomach, small intestine and the large intestine were reduced to percentages of the weight of the entire digestive tube and these percentages plotted on body weight as was done for the parts of the digestive system, and from the resulting curves the proportional parts of the digestive tube were determined and the changes in the proportions of the tube are shown in Figure 10.

The esophagus is merely a tube just long enough to connect the stomach with the terminal end of the pharynx and it decreases from about 10 per cent of the weight of the tube at the beginning of the fetal period to about 4.5 per cent in the largest specimens. The upper area in Figure 10 shows that most of the change in percentage weight of the esophagus occurs in the first 60 grams of body weight and from then on the percentage weight of the esophagus changes very little.

The stomach follows the same general plan. It decreases from about 22 per cent to about 9 per cent of the weight of the entire digestive tube. The most rapid change in its percentage is likewise completed by the attainment of a body weight of 60 grams. It was filled with a clear fluid, probably amnionic fluid in nearly all the fetuses. Windle *et al.* (1939) report finding the amnionic fluid in the stomach of the guinea pig as early as the 42nd day of gestation. In these fetal pups the stomachs were found filled with fluid in the 3.4 gram fetus (56 mm. of body length) and very consistently from then on. Possibly this early distension of the stomach may have something to do with its relatively large size in the smallest fetuses. We have no data on the histological character of the gastric walls.

The major part of the entire tube is, of course, the small intestine.

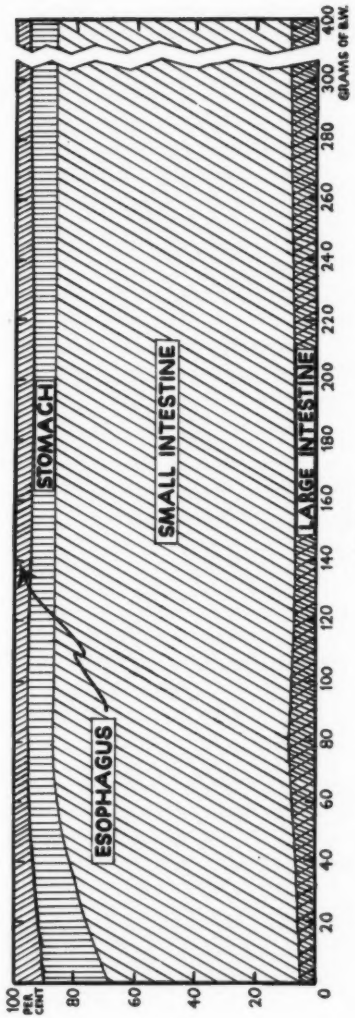

FIGURE 10

Graph showing changes in the percentages of the parts of the digestive tube.

It forms a minimum of about 63 per cent at first and this increases rapidly to about 75 per cent at a body weight of 60 grams and then it increases more slowly to about 78.5 per cent at 100 grams of body weight and remains at this level throughout the remainder of the fetal period.

The large intestine is more irregular in its relative weight. It begins at about five per cent of the entire weight of the digestive tube, increases to about 8.5 per cent at 80 grams of body weight, then decreases to about 7.7 per cent at 140 grams of body weight and then again reaches about 8.5 per cent in the largest fetuses. All of these weights and percentage weights are based on the empty digestive tube. Very little meconium was found in the terminal part of the small intestine but by 90 grams of body weight the terminal part of the large intestine was filled with meconium and by 100 grams of body weight or possibly a little before this, the entire large intestine was filled with meconium. For the next 15 or 20 grams increase in body weight there were patches of meconium to be seen in the terminal part of the small intestine and after about 125 grams of body weight the terminal part of the small intestine was filled for a distance of some few millimeters with closely packed meconium. In one specimen, a newborn male weighing 144.5 grams and with a body length of 164 mm., the large intestine was full of meconium and the stomach was full of coagulated milk.

The rotation of the large intestine was not completed until the fetuses had attained a body weight of about 20 grams. In the specimens smaller than this, the cephalic end of the large intestine was found nearly in the midline of the body and cephalad to the origin of the root of the mesentery of the small intestine. In fetuses of about 20 grams of body weight and above, it had rotated over to the right side in a normal position. It never seems to rotate as far as in man, or the caecum is farther cephalad in the dog at birth than in man.

In general, the esophagus and the stomach decrease from their maximum percentage of the digestive tube weight at the beginning of the fetal period and remain rather constant from 60 to 400 grams of body weight. The small intestine increases from its minimum at the beginning, and the large intestine likewise has its minimum at the beginning of the fetal period. The large intestine is the most variable in the latter part of the fetal period.

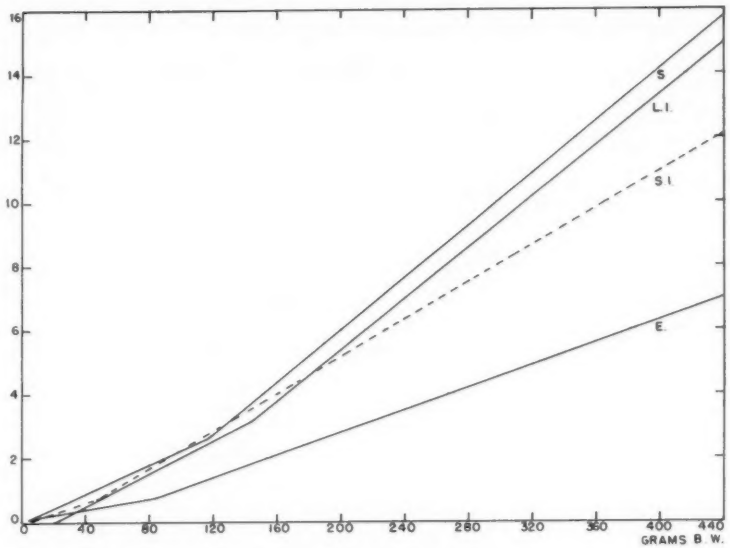

FIGURE 11

Curves showing the growth in weight of the parts of the digestive tube, plotted on body weight. Formulae for all of these curves are given in the text. The numbers in the left margin represent grams for the small intestine and 0.1 grams for the other three parts. S.—stomach, L.I.—large intestine, S.I.—small intestine, E.—esophagus

Parts of the Digestive Tube

The growth in weight in grams of the four divisions of the digestive tube is shown in Figure 11. The distribution of the cases was very much like that of the other parts of the digestive system and so only the curves are shown and thus, with the omission of the individual cases, all four parts can be shown in the one figure. The individual weights were plotted and then the formulae developed as for the preceding figures and these curves were drawn from the formulae. The figures in the left margin represent grams for the weights of the small intestine and tenths of a gram for the weights of the esophagus, stomach and large intestine. Thus the small intestine is the heaviest part, followed in order of decreasing weight by the stomach, the large intestine and the lightest of all, the esophagus.

The formulae from which these curves were drawn are as follows: esophagus, from 10 to 85 grams of body weight,

$Y = 0.00083X + 0.0096$, and from 85 to 440 grams of body weight,

$Y = 0.00175X - 0.0687$; stomach, from 4 to 117 grams of body weight,

$Y = 0.0021X + 0.0097$, and from 117 to 440 grams of body weight,

$Y = 0.0041X - 0.2245$;

small intestine, from 6 to 59 grams of body weight,

$Y = 0.014X - 0.031$, and from 59 to 440 grams of body weight,
$Y = 0.03X - 0.97$;

large intestine, from 20 to 145 grams of body weight,

$Y = 0.0025X - 0.0455$, and from 145 to 440 grams of body weight,
$Y = 0.004X - 0.2630$.

All of these formulae were developed by the method of least squares. The average percentage deviation of the calculated from the observed values are: for the esophagus, 6.29; stomach, 7.09; small intestine, 5.95 and large intestine, 8.51. The percentage deviations show that the curves fit the cases very well. All of these parts grow more slowly in the early part of the fetal period and then all increase more rapidly in the latter part. The esophagus grows the least rapidly throughout the entire fetal period. These curves are very much like those shown for the growth of the parts of the fetal cat digestive tube with one exception. The large intestine in the cat becomes slightly heavier than the stomach.

Figure 12 shows the weights of the four parts of the digestive tube plotted on body length. These curves are all concave superiorly, or the parts increase more rapidly than the body length. The individual cases, as has been described for the preceding figure, were plotted and the curves drawn and the empirical formulae developed by trial and error for these curves. As in the preceding figure the numbers in the left margin represent grams for the weights of the small intestine and tenths of a gram for the other three divisions of the digestive tube.

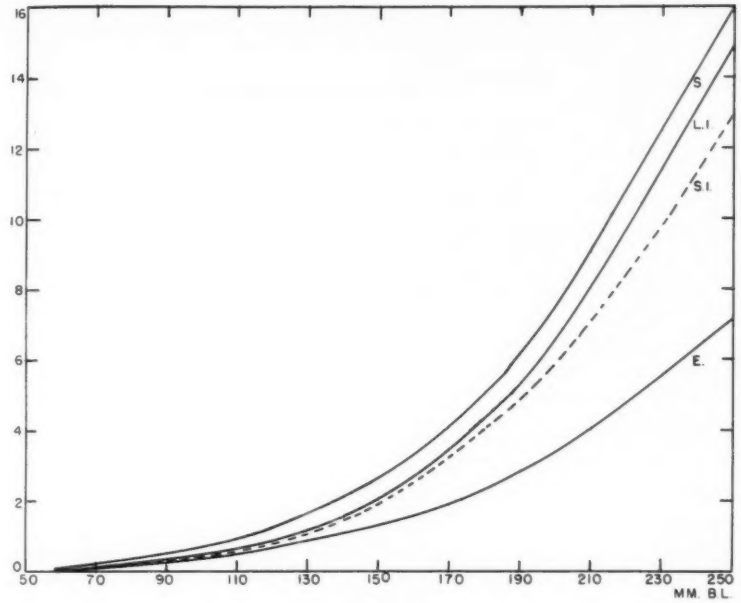

FIGURE 12

These four curves show the growth in grams of the parts of the digestive tube plotted on body length. Formulae for all of these are given in the text. The figures in the left margin represent grams for the small intestine and 0.1 grams for the other three parts. S.—stomach, L.I.—large intestine, S.I.—small intestine, E.—esophagus.

The formulae from which these curves were drawn are given below. Esophagus, from 55 to 250 millimeters of body length,

$$Y = \frac{(0.1X)^{3.4}}{79623}.$$

Stomach, from 60 to 250 millimeters of body length,

$$Y = \frac{(0.1X)^{3.6}}{67223} - 0.0023.$$

Small intestine, from 55 to 157 millimeters of body length,

$$Y = \frac{(0.1X)^{4.6}}{130497},$$ and from 157 to 250 millimeters of body length,

$$Y = \frac{(0.1X)^{3.6}}{8312}.$$

Large intestine, from 60 to 97 millimeters of body length,

$Y = 0.0005X - 0.0287$, and from 97 to 250 millimeters of body length,

$$Y = \frac{(0.1X)^{3.8}}{132526} - 0.0227.$$

The average percentage deviations of the calculated from the observed percentage values are as follows: esophagus, 5.94; stomach, 6.29; small intestine, 3.06, and the large intestine, 6.62. These curves also resemble the similar curves of prenatal growth of the parts of the cat digestive tube, except that when plotted on body length as well as body weight the cat large intestine becomes heavier than the stomach.

Total Increase for Each Part

The growth of each part can be compared with the others by comparing the pitch of the curves or by the size of the function of X in the various formulae. Another method of comparing the growth of each part, or division, is to get the multiples of the heaviest part or organ in terms of the lightest specimen of the same organ or part. To do this the first and last average weights were used. The averages were those used in developing the formulae. This will mean that the total range for all of these is not quite as great as it would be were the lightest and the heaviest individual organ or part compared, but using these averages gives more stable values, not subject to individual variations. These values are shown in Table 1.

The lowest value in this table is 4 times for the increase in body length. Any linear measurement increases less than a measurement of volume or weight and so we would expect this one linear measurement to have the lowest total increase.

TABLE 1

THE TOTAL INCREASES FROM THE FIRST TO THE LAST AVERAGE GROUP FOR THE BODY WEIGHT, BODY LENGTH AND FOR THE VARIOUS PARTS

Body length	4		
Body weight	67		
Liver	59	Digestive tube	187
Digestive system	82	Small intestine	225
Esophagus	85	Large intestine	346
Stomach	87	Pancreas	372

The total body weight increase is lower than all of the others except the increase in the weight of the liver. This means that the digestive system and all of its parts except the liver grow more rapidly in fetal life than the total body growth. In the cat (Latimer, 1948) the digestive tube increases more in the fetal period than the total body weight, but the liver and the pancreas both increase less than the body weight. The liver, as would be expected on account of its changing functions, increases less than the total body weight.

The esophagus and the stomach increase nearly the same amount and also much less than the other parts of the digestive system. The esophagus has little function other than to connect the end of the pharynx and the stomach and it would not have to increase greatly in weight to accomplish this. We might expect a greater increase in the stomach, but it has already been shown that as early as 3.4 grams of body weight, the stomach is well distended with amnionic fluid and the stomach seemed large for the rest of the digestive tract at this time. The increase in the two intestines is to be expected. The pancreas was very small in the earlier fetuses and so its large total increase is to be expected.

SUMMARY

When plotted on body weight, the digestive system and the liver form curves which increase at a constant rate throughout the entire fetal period. The pancreas and the digestive tube form curves which are concave superiorly at first and then form straight line curves throughout the latter two-thirds of the fetal period. The four parts of the digestive tube, or the esophagus, stomach, small and large intestines are each fitted by two rectilinear curves, for all of them increase at a more rapid rate in the latter part of the fetal period.

All of these parts form curves concave superiorly throughout the entire fetal period when these weights are plotted on body length.

The weights of the four parts of the digestive system reduced to percentages of the total body weight and plotted on body weight form curves as follows: the digestive tube and the pancreas increase irregularly at the first and then form straight lines increasing to the largest specimens. The liver forms a straight line decreasing from its maximum at the beginning, while the digestive system forms a straight line which increases from its minimum at the beginning of the fetal period.

These same percentages when plotted on body length are all straight lines. The digestive tube remains at an average of 10.35 per cent throughout the entire period. The percentages of the digestive tube and of the pancreas increase while that of the liver decreases.

Empirical formulae have been developed for all of the above curves except the two irregular percentage curves. This means, that with these two exceptions, either the weight in grams or the percentages of the total body weight of each organ or part can be calculated from either the body weight or the body length.

The digestive tube and the pancreas increase in their percentages of the weight of the entire digestive system and the liver decreases.

The esophagus and the stomach decrease in their percentages of the weight of the digestive tube and the percentages of the two intestines increase.

All of the parts of the digestive system except the liver increase more in weight than the increase in the total body weight. The order of total weight increase during the fetal period is as follows: digestive system, esophagus, stomach, digestive tube, small intestine, large intestine and the greatest total increase of all, the pancreas.

Amnionic fluid was found in the stomachs of the majority of the fetuses beginning with a body weight of 3.4 grams. Meconium was found first in the terminal part of the large intestine, then completely filling it and later on in the terminal few millimeters of the small intestine.

The complete rotation of the large intestine was not completed until a body weight of 20 grams was attained, but after this body weight the caecum was found to the right of the midline of the body.

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PRE-WEANING GROWTH OF THE RAT[1]

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The pre-weaning growth of the rat is an index of the nutritional quality of the mother's diet and it is commonly considered to be a measure of lactation performance. Since the post-weaning growth of the rat has been investigated previously (40), it was considered desirable to undertake comparable growth studies during the pre-weaning period.

The pre-weaning growth of rats was expressed by Brody (11) as curves relating age in days and logarithms of weights in grams and by Zucker *et al.* (248) as curves relating the logarithms of both factors. A similar relation was established for mice by MacDowell et al. (105) over part of the pre-weaning period. Both Zucker and MacDowell, as well as the present authors, have concluded that growth on modern diets is not expressed satisfactorily by Brody's equation.

Zucker *et al.* (247, 248) defined the growth of their rats from birth to weaning at 28 days (post-natal) by the equation

$$\log W = k \log t + \log C$$

where W is weight in grams, t is age in days measured from conception, and k and C are constants. Growth data obtained in Zucker's and the authors' laboratories are plotted on this basis in Figure 1. It will be observed that Zucker's data appear to be represented reasonably well by a straight line whereas those of the present authors are not. Since Zucker *et al.* have concluded that uninhibited pre-weaning growth is denoted by a straight line on a log-log plot, any other type of curve would signify inhibited growth and a sub-optimal diet if this hypothesis were valid. On the basis of this premise, the

[1]The writers' work has been aided by grants from the Nutrition Foundation, Inc., and the University of California. Paper 49. For Paper 48, see Camien *et al.* (39). The post-weaning growth of the rat has been discussed by Dunn *et al.* (40).

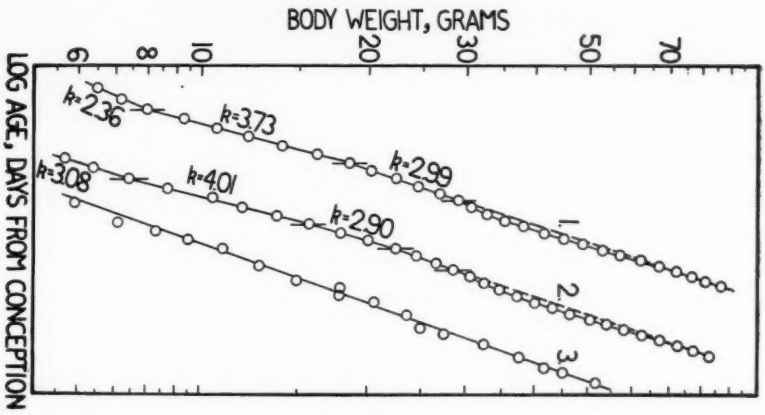

FIGURE 1

Log-log curves showing the relation between body weight and age of suckling male and female rats. The notations are as follows: Curve 1: Male rats, Long-Evans strain, This paper. Curve 2: Female rats, Long-Evans strain, This paper. Curve 3: Female rats, Yale-Sherman strain, Zucker *et al.* (247).

authors' growth data and diets would be inferior and the previous assumption (40) that the Anderson and Smith diet (2, 40, 125) employed in their experiments was near optimal for rats of all ages would be erroneous.

It is not entirely clear, however, whether Zucker's (247) tabulated data represent successive weighings of the same rats throughout the growth period or random samplings at different age levels. Other values given in the same paper (247) for successive weights of rats in the same litter fit a curve (Figure 2-A) of the type similar to that obtained in the authors' experiments. It may be pointed out that many literature data (16, 72, 78, 85, 93, 97, 163, 164, 178, 183, 185, 193, 196) have been selected randomly and, therefore, may be of limited value in determining growth curves even though they may fit particular equations.

It was inferred by Zucker et al. that the divergence from a straight line of the growth data (Figure 2-A) for six young in a litter of eleven (entire litter saved) was explained by a reduction in the quantity of milk provided per young rat from that available normally. These authors concluded, however, that their data adhered to a straight line

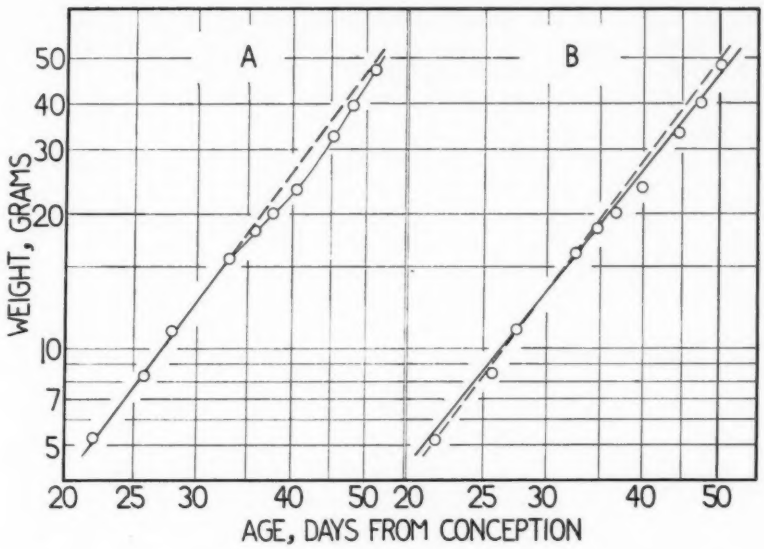

FIGURE 2

Log-log curves showing the relation between body weight and age of six females raised to weaning in a litter of eleven rats. The notations are as follows: A) Solid line: Curve fitted to points given by Zucker *et al.* (248). Broken line: "Normal" curve (Curve 3, Fig. 1) given by Zucker *et al.* (247, 248). B) Solid line: Curve given by Zucker *et al.* (248). Broken line: "Normal" curve given by Zucker *et al.* (247, 248).

although one of lesser slope than normal (Figure 2-B). It appears that this treatment of the experimental data may be an oversimplification tending to obscure a break in the curve at about 35 days of age (Figure 2). If the observed difference resulted because of a decreased milk supply, it might be expected that the divergence would occur *before* this age during the time when the young were dependent solely upon mother's milk. The findings of MacDowell *et al.* (105) with mice and of other workers (25, 96, 123, 178) with rats and mice support this view.

Sherman and Muhlfield (184) found that at 28 days (post-natal) of age the body weight of their rats maintained on Sherman's Diet A varied inversely with the number per litter but that on an improved diet (Diet B) the weight of the young was not affected by the number

in the litter. It was observed by Kozlowska *et al.* (94) that the growth of young rats in litters of five was superior to that in litters of six when the young were restricted to mother's milk with supplements of iron and copper salts. That mice in smaller litters attained greater weights than those in larger litters has been reported by Davenport and Swingle (33), Gates (62) and Crozier and Enzmann (25). The last authors attributed this difference to a reduction in the milk available per young mouse. According to Enzmann (41), the volume of milk production of mice increases with the numbers in the litter, but not in a 1:1 ratio. He observed, however, that the total milk produced decreased during the latter half of the nursing period. Similar decreases in milk volume during the latter half of the lactation period of the rat were found by Cox and Mueller (24). Gaines (61) has suggested that the rate of milk production by cows during advanced lactation is dependent upon factors of a nutritional nature.

In their experiments on mice, MacDowell *et al.* (105) strove for a supply of milk adequate to promote optimal growth of the young. It is of interest that an approximate log-log relationship between body weight and age was maintained up to about 35 days after which there was a marked divergence from a straight line. The curves obtained were similar to the curve shown in Figure 2. It was concluded that this break in the growth curve was independent of the quantity of milk produced although, five years later, Crozier and Enzmann (25) reached the opposite opinion. Numerous investigators (10, 25, 26, 33, 37, 51, 62, 63, 68, 72, 78, 81, 85, 93, 97, 109, 123, 157, 163, 164, 171, 178, 193, 206, 208, 211, 219, 246) have reported rat-growth data which fit log-log curves divergent from straight lines. Straight-line curves with analogous divergencies representing data obtained in the authors' laboratory are shown in Figures 1 and 4-10.

This apparent change in growth rate of the young rat was attributed by MacDowell *et al.* to the "initiation of the natural process of weaning; during this period they take less mother's milk but not their full requirement of solid food. . . . There can be no question but that a new phase of life is inaugurated at the end of the second week by the eating of the first solid food. The transition is not as rapid as that effected by parturition, nor is the change so great as that from uterine to free existence, but none the less it is comparable—a change from one source of nutrition to another." These investigators (104, 105)

assumed that all of the divergencies which occur prior to this natural weaning period are due to environmental influences rather than to inherent growth cycles since "the parabolic curve of the embryo guinea pig continues uninterrupted through stages of development corresponding to those of the suckling mouse."

When the authors' experimental growth data are plotted on Cartesian coördinates (Figure 3), the break formerly observed at 24 days in the log-log curves (Figures 1 and 4-10) disappears. It may be seen, however, that breaks do occur most often at about 29 and 35 days. Although the latter are not invariably pronounced, the regularity of their occurrence argues against the supposition that growth during the entire pre-weaning period can be adequately represented by a continuous smooth curve of the type required by Zucker's growth equation. Lerner (95) has concluded similarly that definite, although small breaks occur in the growth curves of embryos.

Influence of Diet on Pre-weaning Growth. The effect on repro-

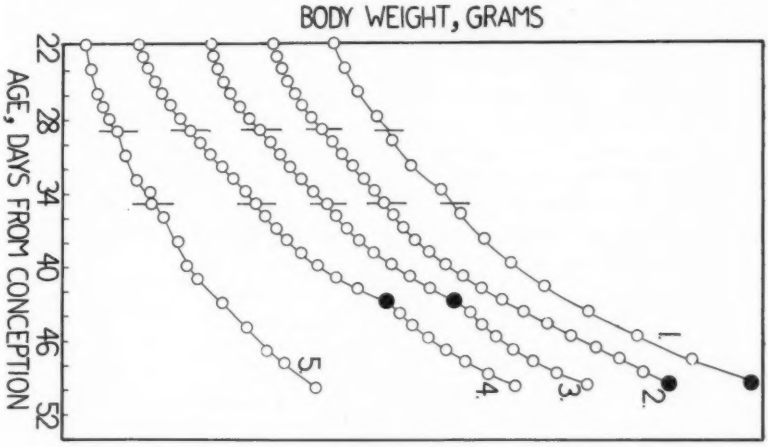

FIGURE 3

Arithmetic curves showing the relation between body weight and age of male and female rats. The notations are as follows: Curve 1. Male rats, Long-Evans strain, Anderson and Smith diet, This paper. Curves 2 and 3. Male rats, Long-Evans strain, Anderson and Smith paste food plus Rockland pellets diet, This paper. Curve 4. Female rats, Long-Evans strain, Anderson and Smith paste food plus Rockland pellets diet, This paper. Curve 5. Female rats, Yale-Sherman strain; curve fitted to data of Zucker *et al.* (247). Closed circles indicate weaning age.

duction and lactation of diets containing different types and levels of proteins and amino acids (1, 17, 18, 26, 27, 32, 38, 60, 70, 71, 74, 81, 94, 100, 107, 111, 118, 119, 122, 130, 154, 160, 174, 177, 191, 192, 200, 201, 220, 245, 246), fats (3, 28, 35, 48, 51-53, 92, 206, 218, 243), vitamins (12, 15, 19, 30, 42-47, 49, 56, 58, 69, 76, 77, 82, 83, 88, 99, 109, 110, 112, 113, 115-117, 127, 128, 131-141, 143, 145-148, 150, 151, 155-157, 159, 161, 168, 199, 202-205, 207-217, 219, 219A, 221-226, 231, 239, 240, 242), minerals (6, 13, 21, 22, 29, 31, 54, 64, 90, 91, 108, 129, 149, 152, 153, 165, 178, 180, 183, 185, 187, 188, 228, 230, 232-234, 236, 237), natural feed rations (14, 75, 167, 179, 182, 184, 186, 190, 194, 198, 227), and purified diets (8, 23, 55, 59, 114, 166, 173, 176, 229, 235, 238) has been studied by numerous workers but in many of the investigations the data reported were insufficient to define growth curves. Reference is made in the present discussion, therefore, only to a few of the more significant studies.

It was found by Mendel and Hubbell (125) that the lactation performance was greatly improved when the Yale-colony rats were fed on a diet consisting of Maynard's (121) calf-meal ration combined with a concentrated (high protein and high fat) paste food. This dietary combination was employed by Anderson and Smith (2) and it has been utilized extensively in the authors' laboratory (40). Maynard and Rasmussen (123) also reported that the efficiency of lactation was increased by increasing the fat content of the calf-meal ration. The effect of different types and quantities of fat in the paste food was studied in the present investigations (Table I).

TABLE 1

COMPOSITION OF DIETARY PASTE MIXTURES

Component	Anderson-Smith Per cent	Anderson-Smith, modified Per cent (M-1)	Anderson-Smith, modified Per cent (M-2)
Whole milk powder	25	27	23.5
Casein, commercial	25	27	23.5
Wheat germ and middlings	20	22	19.2
Lard	30	—	13.0
Wheat germ oil (Viobin)	—	15	13.0
Dried Brewer's yeast	*	9	7.8
Fresh lettuce	**	**	**

*Supplement, 6 gm. per week per rat.
**Supplement, 20 gm. per day per rat.

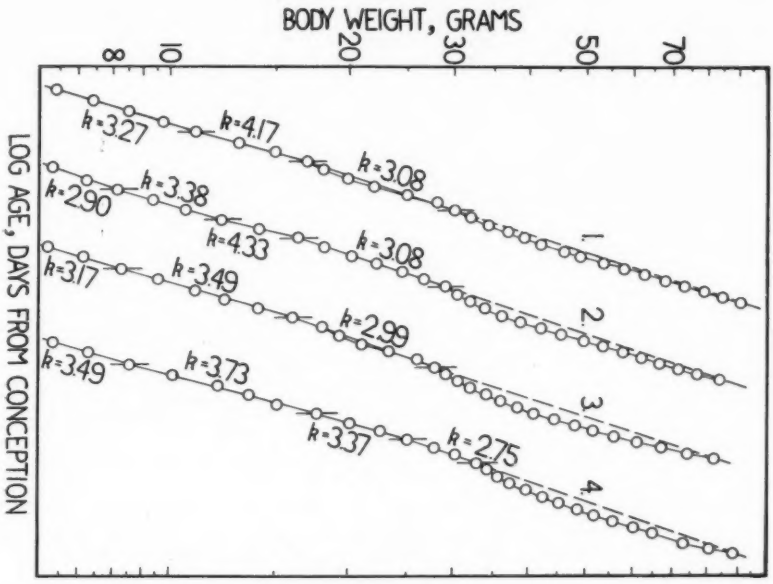

FIGURE 4

Log-log curves showing the relation between body weight and age of male rats of the Long-Evans strain on various diets. The notations are as follows: Curve 1. 30% fat level, Anderson and Smith diet, This paper. Curve 2. 15% fat level, modified Anderson and Smith diet (M-1), This paper. Curve 3. 15+% fat level, modified Anderson and Smith diet (M-1 plus Wheat Germ Oil *ad lib*), This paper. Curve 4. 26% fat level, modified Anderson and Smith diet (M-2), This paper.

As shown in Table II and Figures 4 and 5 the pre-weaning growth curves were similar on the Anderson and Smith diet containing 30 per cent lard, 15 per cent wheat germ oil, 15 per cent plus *ad libitum* wheat germ oil, or 13 per cent lard plus 13 per cent wheat germ oil. The slopes of the growth-weight curves, both for males and females, increased with increasing fat levels in the diets. Although this correlation of fat level and growth weights has been observed by other investigators (20, 35, 50-53, 57, 96, 115, 123, 124, 166, 175, 235), Nelson and Evans (144) have reported recently an inverse relationship between the fat content of purified diets and lactation performance. On the other hand, "lactation index" values of 672, 638, 633, and 667 were found in the present investigations for pre-

TABLE II
WEIGHT OF YOUNG RATS (FIG. 4 AND 5)

Number of litters**	Diet	Conception age, days 22 ♂	22 ♀	24 ♂	24 ♀	29 ♂	29 ♀	35 ♂	35 ♀	43 ♂	43 ♀	50 ♂	50 ♀
4	A & S (30% lard)	6.4	6.0	8.5	8.2	17.0	16.7	29.8	29.8	53.6	53.0	91.0*	88.0*
5	A & S: M-1 (15% wheat germ oil)	6.3	6.0	8.1	7.7	16.3	15.8	28.8	27.5	49.0	45.2	83.0*	75.7*
5	A & S: M-1 (15% W.G.O. plus W.G.O. *ad libitum*)	6.2	6.0	8.2	8.0	16.0	16.0	28.0	28.0	44.3	44.3	82.0*	78.0*
4	A & S: M-2 (13% lard, 13% W.G.O.)	6.3	6.2	8.5	8.2	17.4	16.5	32.2	32.3	51.2	49.0	88.0*	81.0*

TABLE III
WEIGHT OF YOUNG RATS (FIG. 6)

6	A & S: M-1 plus calf meal	6.3	6.0	8.1	7.7	16.3	15.8	28.8	27.5	49.0	45.2	83.0*	75.7*
5	A & S: M-1	5.7	5.5	7.2	7.2	14.8	14.7	25.8	25.6	44.7	44.8	76.3*	73.5*

TABLE IV
WEIGHT OF YOUNG RATS (FIG. 7)

4	A & S plus calf meal	6.4	6.0	8.5	8.2	17.0	16.7	29.8	29.8	53.6	53.0	91.0*	88.0*
4	A & S plus Rockland Pellets	6.5	5.7	8.0	7.4	16.2	15.7	28.9	28.5	52.7	49.8	86.3*	82.1*

TABLE V
WEIGHT OF YOUNG RATS (FIG. 8 & 9)

A—(Fig. 8)

4	A & S plus Rockland pellets	6.5	5.7	8.0	7.4	16.2	15.7	28.9	28.5	52.7	49.8	86.3*	82.1*
2	A & S plus Rockland pellets	6.6	6.5	7.9	8.4	16.5	16.8	30.0	30.2	55.5*	56.6*	82.5	83.0

B—(Fig. 9)

5	A & S: M-1 plus calf meal	6.3	6.0	8.1	7.7	16.3	15.8	29.0	27.5	49.0	45.2	82.9*	75.7*
2	A & S: M-1 plus Rockland pellets	6.3	6.1	8.0	7.5	13.2	12.8	23.8	23.5	45.0*	43.0*	76.1	71.8

TABLE VI
WEIGHT OF YOUNG RATS (FIG. 10)

1[a]	A & S plus Rockland pellets	5.6	——	7.0	——	15.5	——	33.3	——	64.5*	——	93.0	——
2	A & S: plus Rockland pellets	6.6	——	7.9	——	16.5	——	30.0	——	55.5*	——	82.5	——

*Indicates weaning age
**Young reduced to six per litter, usually 3 males and 3 females. Separate mothers, approximately same age (about 16 weeks).
[a]One rat saved per litter.

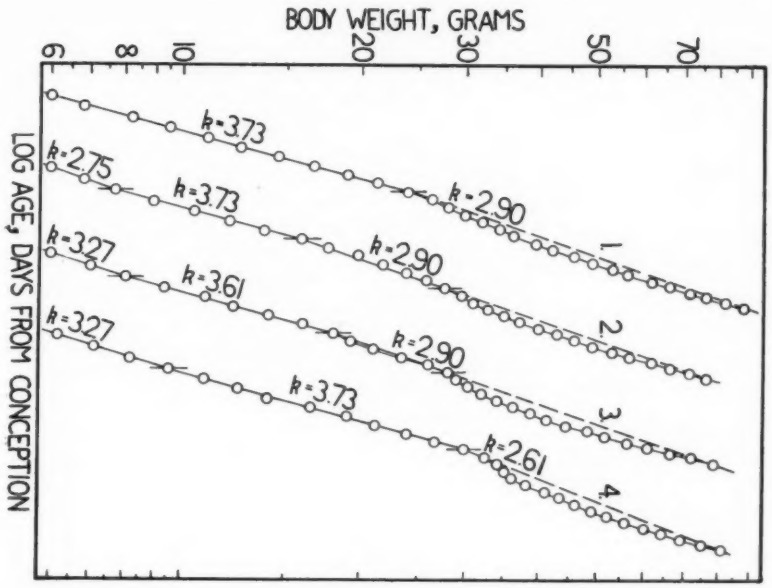

FIGURE 5

Log-log curves showing the relation between body weight and age of female rats of the Long-Evans strain on various diets. The notations are as follows: Curve 1. 30% fat level, Anderson and Smith diet, This paper. Curve 2. 15% fat level, modified Anderson and Smith diet (M-1), This paper. Curve 3. 15+% fat level, modified Anderson and Smith diet (M-1 plus Wheat Germ Oil *ad lib.*), This paper. Curve 4. 26% fat level, modified Anderson and Smith diet (M-2), This paper.

weaning growth at the fat levels in the order stated as calculated from the average log-log slopes for the two periods, 0 to 7 and 7 to 13 days (post-natal). The "lactation index" was defined by Daggs (26) as the sum of two semi-log slopes representing post-natal growth over the periods, 4 to 10 and 10 to 17 days.

In other experiments modified Anderson and Smith diets were employed and the effect on the shape and slopes of the pre-weaning growth curves was studied. Growth data and curves were obtained comparing the Anderson and Smith diet (calf meal plus paste food) with the paste-food component (Table III and Figure 6) and the Anderson and Smith diet with a modified Anderson and Smith diet (Rockland pellets plus paste food) (Table IV and Figure 7). The growth curves were not significantly different.

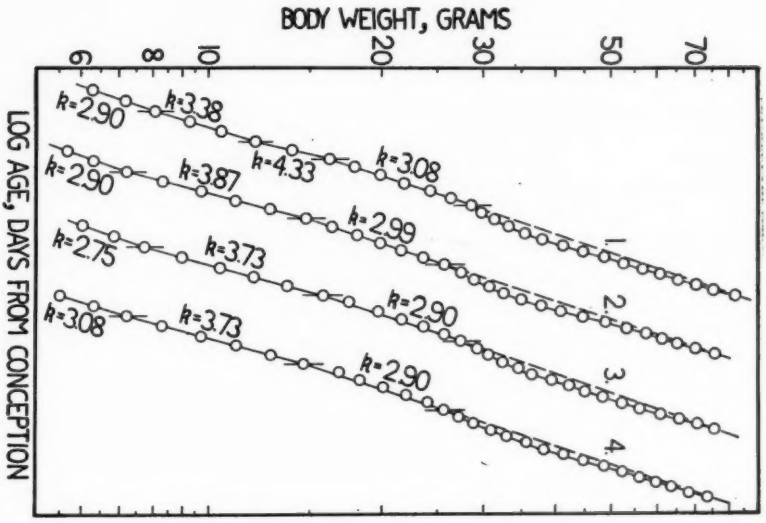

FIGURE 6

Log-log curves showing the relation between body weight and age of male and female rats of the Long-Evans strain on various diets. The notations are as follows: Curve 1. Male rats, modified Anderson and Smith diet (M-1 plus calf meal), This paper. Curve 2. Male rats, modified Anderson and Smith diet (M-1), This paper. Curve 3. Female rats, modified Anderson and Smith diet (M-1 plus calf-meal), This paper. Curve 4. Female rats, modified Anderson and Smith diet (M-1), This paper.

The effect on growth of weaning at three, instead of four, weeks (post-natal) is indicated in Tables V-A and V-B and Figures 8 and 9. The growth curve for young weaned at 21 days appears to diverge from that of young weaned at 28 days. An attempt to wean the young at 15 days (post-natal) was unsuccessful since loss in weight was immediate and marked. Similar observations were made by Bosshardt *et al.* (7) and Enzmann (41) with mice weaned at 15 or 16 days. Although Deuel and Movitt (36) found no significant differences after twelve weeks between the weights of rats weaned at 14 days and those weaned at 21 days, any initial losses and subsequent recoveries would not be apparent in such experiments.

It has been found in the present experiments that reduction of a litter to one male appeared to inhibit lactation and to produce a definite lag in its growth. The results varied from a slight lag in

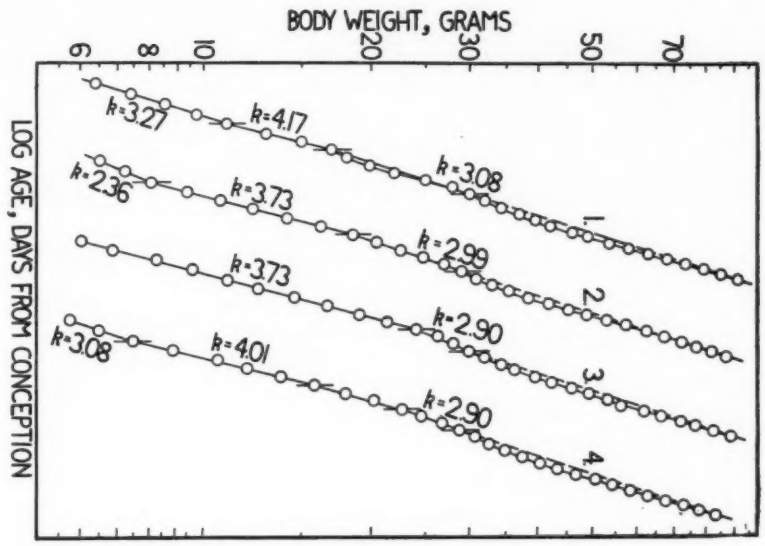

FIGURE 7

Log-log curves showing the relation between body weight and age of male and female rats of the Long-Evans strain on various diets. The notations are as follows: Curve 1. Male rats, Anderson and Smith diet, This paper. Curve 2. Male rats, modified Anderson and Smith diet (paste food plus Rockland pellets), This paper. Curve 3. Female rats, Anderson and Smith diet, This paper. Curve 4. Female rats, modified Anderson and Smith diet (paste food plus Rockland pellets), This paper.

growth to death of the young male rat. In one of the six cases growth apparently was uninhibited (Table VI and Figure 10). The exaggerated effects of litter reduction reported by Parkes (158) on growth of young mice were not observed with mice by MacDowell *et al.* (105), or with rats by the present authors. One result found in the present experiments (Figure 10) appeared to be increase in the slope of the growth curve between the 29th and 34th days and nearly complete disappearance of the divergence from a straight line usually observed at 34 days. It may possibly be found that the divergence from a straight-line growth curve at this age, and perhaps other ages, may be due to differences in food consumption or other environmental factors. Although the influence of environment on lactation has been discussed by Brody (11), Wiesner and Sheard (241),

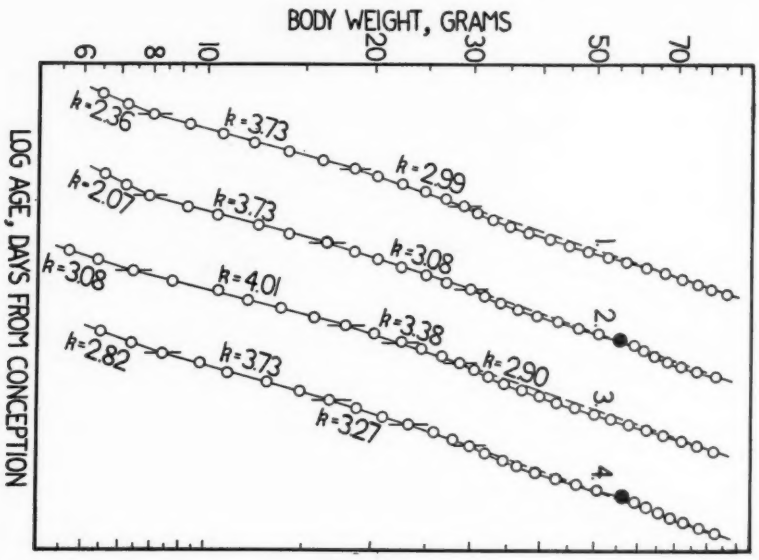

FIGURE 8

Log-log curves showing the relation between body weight and age of male and female rats of the Long-Evans strain weaned at different ages. The notations are as follows: Curve 1. Male rats, weaned at 50 days (post-conception), This paper. Curve 2. Male rats, weaned at 43 days (post-conception), This paper. Curve 3, Female rats, weaned at 50 days (post-conception), This paper. Curve 4. Female rats, weaned at 43 days (post-conception), This paper. Closed circles indicate weaning age. All rats were on a modified Anderson and Smith diet (paste food plus Rockland pellets).

Meigs (124), Peterson (162), Folley (57) and Riddle (169), additional studies are needed.

The probable importance of the volume and the composition of the milk to the growth of the young in litters of different sizes has been emphasized by a number of workers. Cox and Mueller (24) concluded that the volume of milk produced varies during the lactation period by increasing to a maximum at about 16 to 18 days (post partum) and then decreasing until lactation stops. These workers (130) have also shown that the composition of the milk (calcium, phosphorous, magnesium, fat, protein and ash) was nearly uniform during most of the suckling period (8th to 24th day) and that there was little change in these constituents on various diets. Mayer's

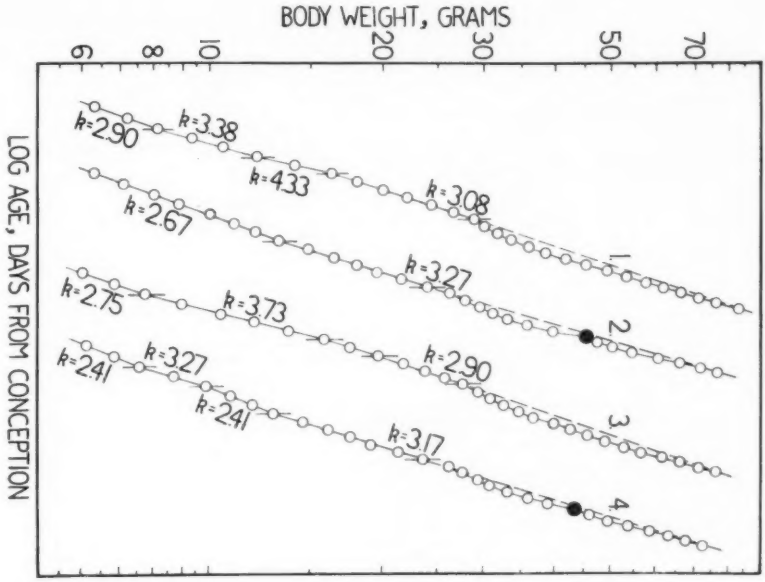

FIGURE 9

Log-log curves showing the relation between body weight and age of male and female rats of the Long-Evans strain weaned at different ages. The notations are as follows: Curve 1. Male rats, weaned at 50 days (post-conception), This paper. Curve 2. Male rats, weaned at 43 days (post-conception). This paper. Curve 3. Female rats, weaned at 50 days (post-conception), This paper. Curve 4. Female rats weaned at 43 days (post-conception), This paper. Closed Circles indicate weaning age. All rats were on a modified Anderson and Smith diet (M-1 plus Rockland pellets).

(120) analysis of rat milk agrees reasonably well with that of the former investigators. Extensive studies on milk production by the mouse have been made by Enzmann (41) who noted that the peak in the curve occurred at about the 12th day of lactation. The quantity of milk ingested as well as the weight lost by the young between suckling periods were determined. Curves constructed from the growth data of Gates (62) and MacDowell et al. (105), and the milk-production data of Enzmann (41), are shown in Figure 11. Although Enzmann did not report data on growth or food (other than milk) consumption, he attributed the weight loss (Fig. 11, curve 2)

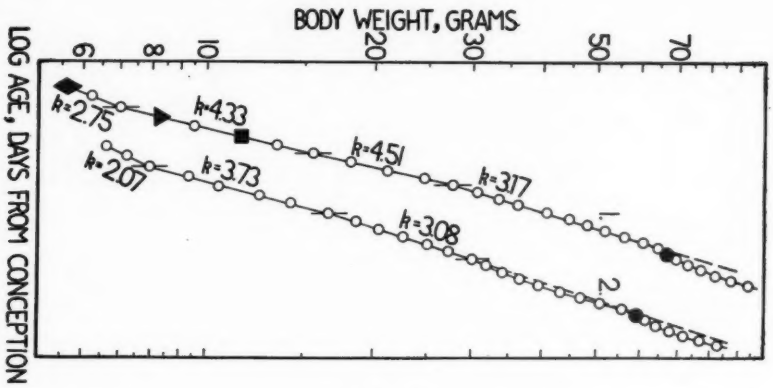

FIGURE 10

Log-log curves showing the relation between body weight and age of male rats of the Long-Evans strain in litters differing in size. The notations are as follows: Curve 1. Male rat, in litter reduced to one pup, This paper. Curve 2. Average weight of males in litter containing 3 male and 3 female pups, This paper. All rats were on a modified Anderson and Smith diet (paste food plus Rockland pellets). Diamond indicates reduction to 6 pups; Triangle indicates reduction to 3 pups; Square indicates reduction to 1 pup; and Closed Circles indicate weaning age (43 days, post-conception).

of his mice to the urine and feces. The marked decrease in weight loss starting on the 14th day was explained by the ingestion of solid food. This conclusion is supported by the observation of Mayer (120) that the stomach contents of suckling rats increased in weight slowly up to about the 12th day and more rapidly for the following 7 days. The curve (Fig. 11, curve 3) showing net weight calculated as the difference between weight loss (Fig. 11, curve 2) and milk consumption (Fig. 11, curve 1) resembles Enzmann's differential growth curve.

The inflections at 7 days in curves 4, 5 and 6 of Figure 11 and at 10 days in curve 2 (Fig. 11) probably may be accounted for by increased activity of the animals or by some internal change in the organism. On the other hand, the break at 13 or 14 days in these curves corresponds closely to the time ingestion of solid food begins. Crozier and Enzmann (25) constructed an ideal growth curve (Fig. 11, curve 5) for one mouse from the values obtained by extrapolating the growth data of litters consisting of from 3 to 12 mice. These authors concluded that "the time curve of the rate of growth (*from*

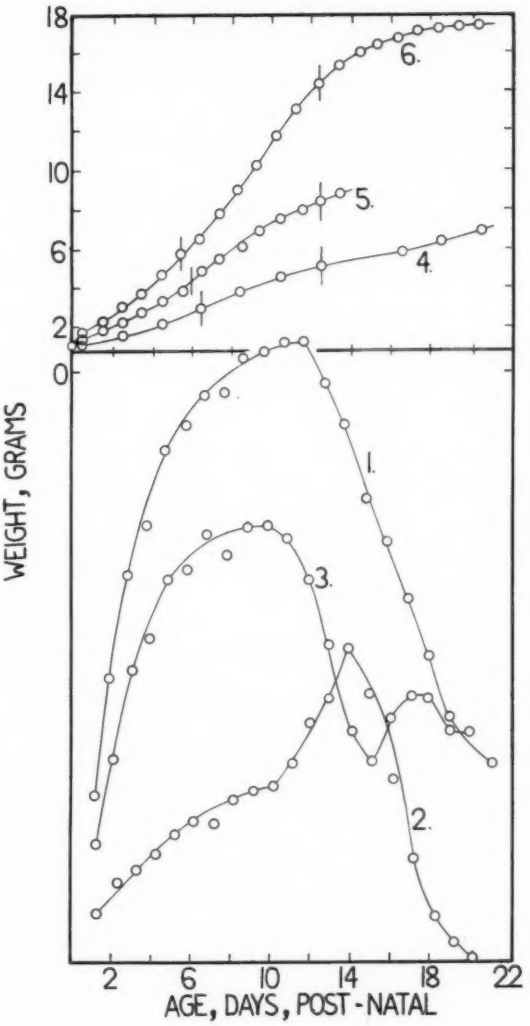

FIGURE 11

Arithmetic curves showing milk consumption, weight loss, and growth of suckling mice. The notations are as follows: Curve 1. Milk consumption, data from Enzmann (41). Curve 2. Weight loss between suckling periods, data from Enzmann (41). Curve 3. Net weight-value of milk consumed (curve 1 less curve 2), This paper. Curve 4. Growth curve, data from Gates (62). Curve 5. Calculated curve of "ideal" growth. Crozier and Enzmann (25). Curve 6. Optimal growth curve, data from MacDowell *et al.* (105).

birth to 14 days, post-natal) then adheres to the capacity for production of milk." Although the growth curves obtained by Kozlowska *et al.* (94) for young rats restricted to mother's milk supplemented with salts differed somewhat from other curves discussed in This Paper, the curves published by these workers were on too small a scale to permit estimation of the data with the desired accuracy. The conclusion of these investigators that young rats could be maintained on mother's milk up to about 60 days (post-natal) with little, if any, increase in weight over that of normal weanlings is in agreement with the observation of MacDowell *et al.* (105). It would be of interest to compare growth under these conditions with that found for young rats on mother's milk and milk supplemented with a near-optimal diet.

Other studies have been made relating to the effect of environment on growth of the young. Slonaker (189) has reported that the average increase in food intake by the mother rat during lactation was proportional to the number of young that were nursed. Increased food consumption of lactating rats was also observed by Wang (238) who quoted John and Schick (87) to the effect that, "the increase in daily food intake during lactation is in proportion to the number of young and parallels the increase in body weight of the new born." The food-consumption curve obtained by Slonaker (189) for lactating mother rats, nursing litters of 7 or more young, resembles the growth curves of pups in litters of this size. That there is a close relation between food intake and milk production during lactation is indicated by the observation of Lusk (98) that milk production is more dependent upon food supply for smaller than larger animals. It has been shown by Sykes *et al.* (227) that mild inanition of rats from weaning to parturition followed by *ad libitum* feeding during lactation had no detrimental effect on the growth of the young.

Growth Cycles. Brody's (9, 11, 248) concept of successive growth cycles is suggested by the curves given in Figure 4. According to Zucker *et al.* (247-249) there are two primary phases, pre-weaning and post-weaning in the growth of the rat. These authors have summarized and discussed the formulations of rat growth and the growth cycles reported by other workers. They have pointed out, in agreement with Gray (66) and other investigators, that theories of cyclic growth based on curves derived empirically by fitting of sub-optimal growth data are not likely to be valid. Zucker *et al.* have stated that, "If one

accepted all of the different possible causes of cycles which have been suggested as definite evidence that all these cycles exist and must be considered, it would have to be concluded that growth is so complex that even an approximately valid quantitative formulation is out of the question. . . . The greatest difficulty in attempts to formulate growth is to avoid letting the general concept of cycles degenerate into a mere adjunct to empirical curve fitting, and if one is inclined to accept many of the proposed cycle causes as probable one might as well give up the idea of ever working out general laws of growth. . . . At the opposite extreme, just as logical, is the position that no cyclic analysis is fully justified, and that therefore none must be made; the data must be fitted whole, or not at all. . . . We feel that an intermediate position, just as logical, but more realistic, would recognize the possibility of cyclic growth but would demand more detailed and objective evidence in support of any *particular* cyclic scheme offered."

The present authors have suggested in a previous paper (40) that there might be a growth phase, in addition to the phases stipulated by Zucker *et al.*, within the primary post-weaning period. As suggested in This Paper, there may be one or more sub-phases in the pre-weaning period. There is need, however, of more detailed and objective evidence to validate any theory of growth of the whole rat and its parts on near-optimal diets. Huxley's classical method has been applied by the present authors to literature data on growth of a part relative to growth of the whole pre-weaning rat or another part. Body parts which have been treated in this manner include the kidney (196), the femur ash (250), total fat (16, 78, 85, 163), protein (16, 85, 163), water (72, 78, 85, 97, 163), ash (13, 16, 78, 163), calcium (6, 85), phosphorous (85), iron (85, 193) and creatine (16). Although breaks occur in the log-log plots of the data reported, few of these authors have endeavored to interpret them in terms of growth cycles. It is of interest, however, that most of the breaks in the curves correspond fairly well to those observed in the growth curves of the entire organism (i.e., at 24, 29, 35, and 50 days, post conception). This conclusion is supported by additional data which have been presented on mortality (mice) (62), pigmentation of adipose tissue (65, 114), activity of reproductive organs to hormones (164), activity of sub-maxillary glands (89), growth of nervous system (93), testis lipids (244), brain cholesterol and cere-

brosides (93, 244), total and essential lipids for whole organism (243), total fat (mice) (126), muscle proteins and enzymes (79, 80), and appearance of ossification centers and skeletal growth (4, 5, 197 and (mice) 67).

It has been suggested by MacArthur and Chiasson (103) that absolute size, rather than age or time, is the factor which determined the breaks in their heterauxetic curves for mice. Acceptance of this interpretation is difficult in view of the observation that similar breaks do occur with animals of different sizes. Green and Fekete (67) concluded from their experiments with mice that, "In early stages, age seems to have more importance than size in differential growth, with the relative rôles later reversed." Jackson (86) and Stewart (195) found that differentiation and development in the young rat proceeded independently of the increase in general body weight.

Although data are insufficient to determine with certainty the cause(s) of the observed breaks in the pre-weaning growth curves of young rats, it seems probable that they may be regulated by internal mechanisms but influenced by environmental factors. It appears somewhat reasonable to assume, however, that the divergence of the pre-weaning growth curve from a straight line at about 35 days (post-conception) is due primarily to a dietary deficiency and becomes accentuated on diets of decreasing nutritional quality.

Summary

It has been shown that growth from birth to weaning of young, Long-Evans rats on the original and modified Anderson and Smith diets did not adhere to a continuous straight-line curve drawn from plots of logarithms of weight and logarithms of age from conception. Four possible phases of growth which were indicated are a) 22 to 24 days, b) 24 to 29 days, c) 29 to 34 days and d) 34 to 50 days. The first phase probably is related to birth weight while the other three phases may represent fundamental changes in growth pattern. The latter may be influenced by the environment especially the quantity and quality of the mother's milk as well as of the accessory food. It may be concluded, tentatively, from the authors' experimental data that the Anderson and Smith diet, while one of the best available, may not be optimal for the growth of young rats during the weaning period. On the other hand, the evidence does not support the con-

cept of Zucker and coworkers that the normal (optimal) pre-weaning growth of the rat follows a straight-line curve of unchanging slope when the growth data are plotted on a log-log basis.

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GROWTH CHARACTERISTICS OF RATS FED A SYNTHETIC DIET

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INTRODUCTION

The use of "synthetic" diets—i.e. diets made of purified major ingredients, and crystallized vitamin supplements has become of late more and more widespread. Such diets have been put to good use in studies of deficiencies of B-complex vitamins. This writer has used them in vitamin A studies, Mayer and Krehl (1948 a, b, c, d, e & f) and Mayer (1948a). They present the obvious theoretical advantage of being of better defined composition and the practical advantage of being perfectly reproducible. However, the following points must also be considered when it is planned to use them:

Synthetic diets are more expensive, as they necessitate the use of expensive crystalline vitamins instead of inexpensive yeast.

They take longer to prepare as all individual vitamin supplements have to be weighed separately.

A fairly extensive preliminary study must be made demonstrating the "adequacy" of the diet as far as the control animals are concerned.

The theoretical risk still exists, when these tests have been fulfilled, that a "borderline" substance has been omitted which is not needed by the normal animal but which is not synthesized in adequate amounts in "abnormal" circumstances. If this additional factor is identified, it permits the beginning of an analysis of the biochemical impairments caused by the stress. If it is not identified, it complicates perhaps unnecessarily the overall picture.

It must be kept in mind that all synthetic diets present, to at least

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the same extent as natural diets—and possibly to a greater extent,—the limitations of having been established, tested and found adequate under certain physiological conditions. Extensions to very different circumstances (for example, life at cold temperature) may prove quite unsatisfactory.

From this argument it follows that in order to derive the full benefit of studies making use of synthetic diets, it is indispensable to first examine the behavior of animals on these rations and to define both their potentialities and their limitations on the diets. As most nutritional work involves the observation of growing animals, an essential point in testing a synthetic diet is its ability to support growth as judged by comparison in this respect with data found in the literature on growth on "normal" diets. Other criteria may be explored whenever circumstances demand it.

The data, comparisons and discussions which follow refer to groups of animals fed the "reference" synthetic diet A_1. The general results and conclusions reached are nevertheless valid for a variety of widely different diets (used by the writer in vitamin A studies) which differ from diet A_1 by the isocaloric replacement of carbohydrate, protein and fat (the protein level being maintained, in all combinations, above the minimum necessary for acceptable growth), This study may therefore be considered as presenting a fairly general significance.

Method

Experimental animals and conditions; composition, adequacy and limitations of the diet.

The experimental animals belonged to two strains: Sprague-Dalley and Connecticut Agricultural Experiment Station or "Yale".

They were invariably started on the synthetic diet at weaning. Both males and females were used in all experiments. All animals were weighed every four days.

All animals were kept in individual, screen-bottomed cages, at constant temperature (75° F.). Hygrometry was also maintained constant (66%). The animals were exposed 12 hours to artificial light and 12 hours to darkness in the course of every 24 hours.

The diet used in the studies reported here had the following composi-

tion:[1,2] Casein ("vitamin-free" Labco) 25%, refined corn oil 5%, salts mixture[3] ("Salts IV & cobalt") 4%, crystalline sucrose 65.7%, cystine 0.2%, choline 0.1%.[4] The following vitamin supplements were used, per kilo of diet: E(α-tocopherol) 100 mg., K(2 methyl-1-4 Naphtoquinone) 5 mg.; D_2 ("viosterol") 100 mg., dissolved in corn oil; thiamine 5 mg.; pyridoxine 5 mg.; riboflavin 5 mg.; nicotinic acid 50 mg.; pantothenic acid 50 mg.; biotin 0.5 mg.; folic acid 0.5 mg.; inositol 100 mg.; para-aminobenzoic acid 100 mg.; the water-soluble vitamins being dissolved in 50% ethanol and the solution dried on casein. In addition, all animals were given orally 1,200 I.U. of vitamin A every ten days.[5]

Diet A_1 besides being, as will be presently demonstrated, perfectly adequate for good growth was found adequate for reproduction: 5 couples of "Yale" rats fed diets A_1 since weaning were found capable of reproducing; litters, normal both in number and in weight, were obtained. Experimental data are however too incomplete to allow for any conclusion as to the adequacy of this diet as far as lactation is concerned.

A brief study of the adequacy of this diet for life and growth at low temperature was undertaken. Ten growing "Yale" strain male and female rats fed diet A_1 were placed in a cold room (7° C.). They showed poor growth and occasional profuse hemorrhages of the tail. Administration of ascorbic acid (by 50 mg. intraperitoneal injections) improved the hemorrhagic condition[6] but did not check it altogether. It seems that substances not included in diet A_1 may be required at cold temperature in addition to ascorbic acid; perhaps, in particular, antithemorrhagic factors like citrin (Bentsath, Rusznyak

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[1]The writer is indebted to Doctors W. A. Krehl and P. F. Fenton for advice regarding the establishment of the diet.

[2]This diet was planned to be used in studies involving both rats and mice. Liberal amounts of the vitamins known to be required by either species were therefore included.

[3]The salt mixture used, a modification of the mixture described by Philipps and Hart (1935) consisted of: $CaCO_3$, 600 g; K_2HPO_4, 645 g; $CaHPO_4$, 124 g; $MgSO_4$, $7H_2O$, 204 g; NaCl, 335 g; $Fe(C_6H_5O_7)_3$, 0.5 g; $CuSO_4 \cdot 5H_2O$, 0.6 g; $CoCl_2$, $6H_2O$, 0.6 g.

[4]Cystine and choline, although probably not essential at the 25% level of casein used in A_1, were added so as to make possible the eventual isocaloric replacement of casein by sucrose down to a 10% casein level without compromising the adequacy of the diet for growth.

[5]In later studies, when vitamin C was added to the diet (Mayer and Krehl, 1948 d), the proportion was 1 gm. of ascorbic acid per kilogram.

[6]Therien and Dugal (1947) have shown that guinea pigs present an increased requirement for ascorbic acid when placed in the cold.

and Szent-György, 1936) although they have never been demonstrated to be required by the rat under "normal" conditions may, like ascorbic acid[7] become the limiting factor at times of stress.

Growth Study

Four groups of animals were used: "Yale" strain males, 10 animals; females, 6 animals; Sprague-Dawley males, 6 animals; females, 6 animals. Weights were recorded every 4 days.

The results obtained are summarized in Tables I and II. A comparison of the growth rate obtained in the experiments reported here,

TABLE I
Comparison of Weights of "Yale" Strain Male Rats Fed Diet A_1 and Classical "Natural" Diets at 7 and 15 Weeks

Authors[1]	Weight at 7 weeks (gm.)	Weight at 15 weeks (gm.)
Mendel and Hubbel (1919)	80	180
Mendel and Hubbel (1925)	90	200
Mendel and Hubbel (1935)	190	360
Anderson and Smith (1932)	200	440
Present Results[a]	274	506

[1]All data except present results quoted from "Optimal Growth of the Rat", M. S Dunn, E. A. Murphy and L. B. Rockland (1947).
[a]Ten animals in the group.

TABLE II
Comparison of Rates of Growth of "Yale" and Sprague-Dawley Rats Fed Diet A_1 (Control Animals)

Age (Weeks)	"Yale" Males	"Yale" Females	Sprague-Dawley Males	Sprague-Dawley Females
3	47.8	50	50	50
5	134.9	129.4	109.0	105.0
7	273.5	186.6	200.6	153.5
9	352.2	212.6	279.0	179.8
11	418.1	242.1	303.8	200.0
13	481.1	262.1	352.8	230.0
15	506.1	272.1	366.0	——

"Yale" males 10 animals; other groups 6 animals.

using synthetic diet A_1, with classical data (Table I) obtained with "natural" diets makes it obvious that diet A_1 allows for quicker growth than had been previously reported. An attempt for a more objective comparison was made by fitting the data to a growth equation and comparing the parameters obtained with published data. However,

[7]Mayer and Krehl (1948 b).

the following difficulties were encountered: More than a dozen equations can be found in the literature which claim to be representative of the function: weight = F (t) for the rat. Backman (1931), Hatai (1911), Gompertz (1932), Brody (1925), Robertson (1907-8), Crozier (1926), Pearl (1924), von Bertalanffy (1934), Fischer (1928), von Hoesslin (1926-27), Wetzel (1932-33) have all proposed different equations. These equations have conclusively been shown by Zucker and Zucker (1941) to be representative of growth under suboptimal nutritional conditions. The latter authors pointed out that unless nutrition was eliminated as the limiting factor a growth curve could not be considered to be a "pure expression of the inherent growth characteristics of the organism." They also emphasized the fact that many of the equations found in the literature had been obtained by the repeated working over of the same old—and unsatisfactory—data. On the basis of their own data on the growth of normal albino rats, they were led to propose as "growth" equation:

$$\ln \frac{A}{w} = \frac{k}{t} \qquad (1)$$

where w is the weight at the age t, A is the "limit" weight of the species and k a characteristic factor. Previous data were shown to fit this equation, which has been accepted by later authors as representative.[8] Consideration of weights reached on synthetic diet A_1

TABLE III

COMPARISON OF VALUES OF k (ZUCKER'S EQUATION: $10g\ w = \log a - \frac{k}{t}$), FOR "YALE" STRAIN MALE RATS FED NATURAL DIETS AND FED DIET A_1

Authors[1]	k
King (1915)	3.73
Macy *et al.* (1927)	3.66
Smith and Bing (1928)	3.67
Maynard (1930)	3.83
Freudenberger (1932)	3.70
Sperry and Stoyanoff (1934)	3.68
Mendel and Hubbel (1935)	3.73
Zucker and Zucker (1941)	3.65
Present Data (1947), Average Value[a]	2.50

[1]All values of k, except present data, calculated by Zucker and Zucker (1941-42) from original data.
[a]Ten animals in group.

[8]See in particular the review "Optimal Growth of the Rat" by M. S. Dunn, E. A. Murphy and L. B. Rockland (1947).

and of the corresponding values obtained for Zucker's coefficient (Tables III, IV and V) show that not only does this diet allow for a quicker overall growth (and hence for a smaller average k) than had been previously recorded, but also that Zucker's relation does not hold under these conditions. Further, the discrepancy seems to be

TABLE IV

WEIGHT OF "YALE" STRAIN MALE RATS FED DIET A_1 AS A FUNCTION OF AGE[1] CORRESPONDING VALUES OF ZUCKER'S COEFFICIENT[2] (Ten Animals in Group)

Age (Weeks)	Weight (gm.)	Log weight	$k = t \log \frac{A}{W}$ (Zucker's Coefficient)
3	47.8	1.67943	3.38
5	134.9	2.12808	3.39
7	273.3	2.43648	2.59
9	352.2	2.54679	2.33
11	418.1	2.62128	2.03
13	481.1	2.68224	2.30
15	506.1	2.70424	1.52

[1]Age counted from birth; weights determined from weaning (at 3 weeks).

[2]$\log w = -\frac{k}{t} \log A$ where w = weight; k = constant; t = age in weeks; A = limit weight (taken here as 640 gm.).

TABLE V

WEIGHT OF MALE SPRAGUE-DAWLEY RATS FED DIET A_1 AS A FUNCTION OF AGE[1] CORRESPONDING VALUES OF ZUCKER'S COEFFICIENT[2] (Six Animals in Group)

Age (Weeks)	Weight (gm.)	Log weight	$k = t \log \frac{A}{W}$ (Zucker's Coefficient)
3	50	1.69897	2.80
5	109.0	2.03743	3.97
7	200.6	2.30233	2.32
9	279.0	2.44560	1.69
11	303.8	2.48259	1.66
13	352.8	2.54753	1.12
15	366.0	2.56348	1.05

[1]Age counted from birth; weights determined from weaning (at 3 weeks).

[2]$\log w = -\frac{k}{t} \log A$ where w = weight; k = constant; t = age in weeks; A = limit weight (taken here as 430 gm.).

due essentially to the reason which Zucker and Zucker had shown to be responsible for the inadequacy of previous equations—namely, the fact that "better" nutritional conditions produce not only an average increase in the growth rate but also an improvement in the condition of the animal which "builds up," so that the discrepancy increases as growth proceeds. The form of the function, not only the slope of the curve, is progressively modified, the modification becoming more important as the still growing animal has been longer on the diet.[9]

The mechanism of the initiation of this discrepancy is as yet not explained; two interpretations seem plausible; the first is that diet A_1 contains extremely liberal amounts of all B complex vitamins, including some factors the necessity of which has not been demonstrated in the Nutrition of the Rat but which may have an accessory effect on growth; the second is that the diet being a dry powder without any roughage presents to the very young rat a food of high energy content (4 calories per gram), high protein content and readily assimilable at a period when the small capacity of the gastrointestinal tract may be a limiting factor.

Conclusion

Several important lessons may be derived from these experiments. The first one is that it is now possible to devise synthetic diets which will insure "better," i.e. quicker growth in the Rat than previous "natural" diets used; in this respect it may be added that some diets other than A_1, also used by the author, have permitted even quicker growth (higher levels of dietary fat and—at least during the first weeks after weaning—higher levels of dietary proteins afford an even greater acceleration).

The second is that rapidity of growth is not in itself a sufficient criterion of the general adequacy of the diet; diet A_1, for instance, although "better" from a growth standpoint than any "natural" diet studied does not permit growth in the cold and may be inadequate for the support of good lactation. This may be important in judging human diets from the point of view of Nutrition in Public Health.

[9]This point seems particularly important as it may well be that the rapidity of the growth obtained on the synthetic diets used here may be partly responsible for the acuteness of the symptoms observed in studies of deficiencies, particularly when the deficiency has its most definite effects at the period of maximum growth. (Mayer and Krehl, 1948 d).

The third is that the establishment of equations of a classical type expressing "growth" as a function of time seem quite illusory and that these functions are likely to prove worthless every time a serious improvement in the diets is realized. This does not however, mean that satisfactory "growth" functions cannot be found, but rather that "growth" has to be defined in a more accurate and significant fashion. In all the studies mentioned in this article, growth was taken as identical with an increase in weight. From a thermodynamical point of view, however, such an increase may have widely different meanings: an increase by 5 gm. of a very young animal represents approximately 1 gm. of protein and 4 gm. of water and mineral salts, or an energy content of 4 to 5 calories. An increase by 5 gm. of an older animal represents 5 gm. of fat or 45 to 50 calories. The introduction of such thermodynamical considerations in the establishment of "growth" equations will be the subject of another article by the author, now in preparation, and which, it is hoped, will put this question on a more rational basis.

Summary

The characteristics of the Growth of two strains of rats fed a synthetic diet have been studied. It is shown that the speed of growth is greater than any other growth recorded in the literature for rats fed natural diets. Furthermore, the character of this growth is such that it cannot be represented by classical growth functions, even the most favorable existing so far, such as Zucker and Zucker's. The reasons for such a discrepancy are discussed and a new basis is suggested which will permit the establishment of an entirely different type of growth functions.

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